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Molecular Modeling of Novel Tryptamine Analogs with Antibiotic Potential Through Their Inhibition of Tryptophan Synthase

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Molecular Modeling of Novel Tryptamine Analogs with Antibiotic Potential Through Their Inhibition of Tryptophan Synthase

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Abstract

The growing prevalence of antibiotic-resistant bacteria is a global health crisis that threatens the effectiveness of antibiotics in medical treatment. Increases in the number of antibiotic-resistant bacteria and a drop in the pharmaceutical development of novel antibiotics have combined to form a situation that is rapidly increasing the likelihood of a post-antibiotic era. The development of antibiotics with novel enzymatic targets is critical to stall this growing crisis. *In silico* methods of molecular modeling and drug design were utilized in the development of novel tryptamine analogs as potential antibiotics through their inhibition of the bacterial enzyme tryptophan synthase. Following the creation of novel tryptamine analogs, the molecules were analyzed *in silico* to determine their binding affinity to human MAOB and the *E. coli* α-subunit, *E. coli* β_2-dimer and the *M. tuberculosis* β_2-dimer of tryptophan synthase. Ten tryptamine analogs displayed significant increases in tryptophan synthase binding affinity and show promise as potential antibiotics and antibiotic adjuvants. Further *in silico* modeling determined that the binding sites of the tryptamine analogs were similar to wild-type tryptamine in the *E. coli* β_2-dimer, the *M. tuberculosis* β_2-dimer and human MAOB, while the analogs' binding sites to the *E. coli* α-subunit differed. Although no tryptamine analogs increased tryptophan synthase binding affinity while decreasing human MAOB binding affinity, related increases in MAOB binding affinity warrants further research into the analogs' potentials as MAO inhibitors. Given the increases in tryptophan synthase binding affinity and similar β_2-dimer binding sites, a provisional patent was filed on the ten identified tryptamine analogs. Moving forward, we recommend the synthesis of the ten identified tryptamine analogs. Following synthesis, further research should be conducted to determine the *in vitro* and *in vivo* antibiotic properties of the ten tryptamine analogs.

Introduction

The Arms Race – Bacterial Evolution of Antibiotic Resistance

The pseudo-competition between human development of new antibiotics and the subsequent evolution of different antibiotic resistant bacterial strains is akin to an arms race. As humans develop a novel antibiotic to combat antibiotic-resistant strains of bacteria, the competition induced by this new antibiotic and subsequent bacterial evolution results in strains of bacteria that are resistant to the new antibiotic. Those antibiotics with the adaptive traits to counteract the mechanisms of the antibiotic outcompete the non-resistant bacteria and subsequently produce more antibiotic-resistant bacteria.

Bacteria can attain antibiotic resistance through two general methods. First, the bacteria may acquire a spontaneous genetic mutation through the reproduction process that yields resistance to the antibiotic in question. Although such a random mutation is exceedingly rare, the rapid speed of reproduction and massive number of bacterial organisms allows for such a resistance-causing mutation to occasionally occur. Second, a bacteria organism may acquire antibiotic resistance from other bacteria through three methods of horizontal gene transfer. During the process of conjugation, the bacteria come into direct contact with another and transfer genetic material directly between the two bacterial organisms. Bacteria can also perform transformation where they uptake exogenous DNA fragments from their surrounding medium. Finally, in the process of transduction a bacteriophage virus acts as an intermediate for the transfer of genetic material. The bacteriophage uptakes bacterial chromosomal DNA into the head of the virus and this genetic material is subsequently transferred to other bacteria the virus infects. All three

aforementioned methods of horizontal gene transfer allow for the transfer of antibiotic-resistance mechanisms from one bacteria to another if the shared genetic material contains a gene that encodes for antibiotic resistance.

Once a bacterial strain acquires antibiotic resistance these bacteria maintain a selective advantage over their non-resistant counterparts in the presence of the antibiotic in question. When this antibiotic is introduced the non-resistant bacteria are killed off with relative ease and the bacteria that remain are those with the antibiotic-resistant gene. Even if only one bacterium has acquired antibiotic-resistance, that bacterium is able to rapidly reproduce and create entire colonies of bacteria with the same antibiotic-resistant gene after the competition from the non-resistant counterparts is eliminated. A recent study reported that in 2012 approximately 70% of bacteria that cause human infections were resistant to at least one of the drugs that are commonly used to fight them (Bax and Griffin, 2012).

To eliminate the bacterial colonies that developed resistance to one antibiotic, a second antibiotic must be introduced. While this may not present a serious issue if the bacterial colony is only resistant to a single antibiotic, it is possible, and increasingly more prevalent, for bacteria to be resistant to multiple antibiotics. Multiple-drug resistance (MDR) may occur both within a single family of antibiotics and across multiple antimicrobial families. Cross-resistance occurs when a bacterial strain is resistant to multiple antibiotics in the same family and is usually the result of a single resistance mechanism that inhibits the function of multiple alike antibiotics. Bacterial MDR across multiple antibiotic families usually occurs as the result of a combination of different independent mechanisms of resistance coded for by individual genes. Although these independent mechanisms of resistance are coded for by different genes, several of these genes are oftentimes present on a

single plasmid that then may be transferred to bacteria through a single act of horizontal gene transfer. When a bacterium receives or takes up this plasmid these multiple individual mechanisms of resistance are then able to coexist within the bacteria organism, resulting in MDR bacterial strains that present resistance across multiple antibiotic families (Brown, 2010). The most common example of MDR bacterial strains from multiple individual resistance-mechanisms is methicillin-resistant *S. aureus* (MRSA), a bacterial strain that is resistant to almost all known antibiotic agents as the result of multiple different mechanisms (Nue, 1992).

Overall, the bacterial development of antibiotic resistance to the antibiotic agents that humans discover and develop is inevitable. Gene D. Wright summarizes this inevitability when he explains:

> Bacteria are ancient organisms that have adapted to virtually all environmental challenges on the planet. They live in environments dominated by small molecules and have evolved both specific and nonspecific mechanisms to evade or detoxify noxious compounds including antibiotics…There are no irresistible antibiotics. (Wright, 2012: 25)

Regardless of the antibiotic, bacterial strains will eventually develop mechanisms that infer resistance to the drug molecule. Bacteria have found ways to survive for millions of years and they will undoubtedly continue to survive for millions of years more. Earlier research noted that a recent database lists more than 20,000 potential resistance genes of approximately 400 types found in available bacterial genome sequences (Davies and Davies, 2010). Therefore, to combat this continual development of antibiotic resistance humans need to continually develop novel antibiotics to which bacteria have yet to develop a resistance.

Antibiotic Drug Discovery – From Penicillin to the "Golden Age"

In 1928 Dr. Alexander Fleming discovered the first antibiotic from the mold *Penicillium notatum* and mass production of penicillin began in the 1940's. In 1941 all strains of *Staphylococus aureus* were susceptible to penicillin but by 1944 particular strains of *S.aureus* were able to destroy penicillin (Manfredi, 2009; Neu, 1992). Less than fifty years later researchers observed that over 95% of *S. aureus* strains were resistant to penicillin and other similar antibiotics (Nue, 1992). While the development of penicillin resistance was the first such example of a bacteria developing antibiotic resistance, it was not unexpected. In his 1945 Nobel Prize Speech Dr. Fleming noted, "It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body" (Fleming, 1945: 93). Dr. Fleming predicted that, although his discovery was one of the most significant of the 21st century, its clinical usefulness would begin to decline as bacteria developed resistance to the drug through evolutionary pressures.

After the widespread success of penicillin Dr. Selman Waksman and his cohorts began screening soil microbes for potential new antibiotic agents. In 1943 Waksman and his graduate assistant Albert Schatz discovered Streptomycin, which successfully killed many gram-negative bacteria, most importantly *Mycobacterium tuberculosis*, the bacteria responsible for tuberculosis infections (Gottfried, 2005; White, 2011). Unfortunately, the usefulness of Streptomycin was limited by its toxic side effects, most notably ototoxicity (White, 2011). However, the work of Dr. Waksman's lab in identifying soil microbes as potential sources of antibiotic agents coupled with the widespread and continued success of

penicillin led to what is known as the "golden age of antibiotic discovery" (White, 2011; Davies, 2006).

The widespread commercial success of penicillin, and to a lesser degree streptomycin, soon led to a significant investment in antibiotic research from the pharmaceutical industry. Every major pharmaceutical company instituted major screening programs in an effort to discover their own antibiotics. The vast majority of current antibiotic classes were discovered during this "golden era" period spanning from the late 1940's to 1960's (White, 2011; Silver, 2007). By 1952, antibiotics existed to treat infections from both gram-positive and gram-negative bacteria (Gottfried, 2005). In only a ten-year period spanning from 1950-1960 approximately half of the antibiotics commonly used today were discovered (Davies, 2006). The impact of this period of immense antibiotic discovery simply cannot be understated. The development of antibiotics over this golden era is credited with playing a central role in the transition of worldwide medicine from an "age of pestilence" to the modern "age of degenerative [chronic] diseases" (Gottfried, 2005: 10). In 1936, before the discovery of penicillin and other antibiotics, approximately 280,000 Americans died of bacterial infections. Less than two decades later in 1952 this number dropped to 95,000 (Gottfried, 2005). Using population estimates at the time that drop correlates to a decrease in the bacterial infection death rate from 216/100,000 in 1936 to 59.7/100,000 in 1952 (Gottfried, 2005). Over this same 16-year period the life expectancy at birth increased an extraordinary 10.1 years, from 58.5 years in 1936 to 68.6 years in 1952 (Gottfried, 2005). This increase in the average life expectancy of Americans over that 16-year period is inevitably not entirely due to the rise of antibiotics in medical treatment. However, the effect of antibiotics was undeniably significant, especially when considering that in the 50 years

following 1952 the life expectancy at birth only increased another 8.7 years to 77.3 years by 2002 (Gottfried, 2005).

The implementation of penicillin and other antibiotics as a foundation of medical treatment has saved countless lives. However, recent developments in antibiotic resistance and a collapse in the development of new antibiotics have begun to threaten the worldwide effectiveness of antibiotic treatments. Many researchers worry that, unless things change soon, humans may soon be faced with a post-antibiotic era. Shockingly, Dr. Fleming predicted this crisis a 1946 New York Times article when he stated:

> The public will demand [the drug]…then will begin an era…of abuses. The microbes are educated to resist penicillin and a host of penicillin-fast organisms is bred out which can be passed to other individuals and perhaps from there to others until they reach someone who gets a septicemia or a pneumonia which penicillin cannot save. In such a case the thoughtless person playing with penicillin treatment is morally responsible for the death of the man who formally succumbs to the infection with the penicillin-resistant organism. I hope this evil can be averted. (Fleming, 1946; Bartlett et al., 2013)

Over 50 years ago Dr. Fleming warned against the evil of antibiotic abuse and the subsequent rise in antibiotic resistance. However, these warnings appeared to have fallen on deaf ears as today the public faces an undeniable global health crisis of rapidly increase antibiotic-resistant bacteria coupled with an untimely and potentially devastating decrease in new antibiotic discovery and production.

The Public Health Crisis

The appearance of antibiotic-resistant bacteria is currently a major public health crisis that demands the attention of the scientific community. A 2016 report estimated that in the United States two million infections a year are caused by bacteria resistant to at least one

antibiotic (O'Neill, 2016). They approximated that the treatment of these antibiotic-resistant strains cost the US health care system 20 billion USD in excess costs (Medina and Pieper, 2016). A 2013 study found that at least 23,000 of these two million individuals die as a direct result of their antibiotic-resistant bacterial infections and many more die from complications induced by these bacteria (CDC, 2013). MRSA alone is responsible for almost 11,285 deaths per year in the United States (Gross, 2013). Multiple studies estimated that worldwide 200,000 people a year die from multi-drug resistant strains of tuberculosis (TB) alone (Gross, 2013; CDC, 2013; RAR, 2016). A national survey of infectious disease specialists in 2011 found that 63% of the surveyed specialists had seen an untreatable bacterial infection resistant to all available antibiotics in the past year (Hersh et al., 2012 Spellman and Gilbert, 2014). Author Ron Daniels writes that "increasing bacterial resistance to common antibiotics may put an 'end to modern medicine as we know it' (Daniels, 2012: 11).

This growing state of crisis at the hands of antibiotic-resistant bacteria is the result of an aggregation of several issues that combine to result in the overuse of antibiotics in the medical and agricultural industries. This overuse of antibiotics allows increased opportunity for antibiotic-resistant bacterial strains to flourish and multiply in antibiotic filled environments where their resistance-genes grant a selective fitness advantage. The first of these issues is the general over prescription and overuse of antibiotics in the medical field. Over prescription and overuse of antibiotics is a worldwide issue, however it is especially prevalent in the United States. Past research found that individuals in the US consumed an average of 22 antibiotic pills per year (Boeckel et al., 2010). In many states the number of prescribed courses of one antibiotic, whereas one course represents an entire antibiotic

treatment, exceeded the population (Gross, 2013; Ventola, 2015). Therefore in these sates individuals on average received more than one antibiotic treatment course per year. Worldwide the overuse of antibiotics is a worsening issue, with the worldwide antibiotic consumption increasing 36% in the ten-year period from 2000-2010 (Boeckel et al., 2010; Morrill and LaPlante, 2015). The issue of antibiotic overuse is exacerbated by the widespread inappropriate prescribing of antibiotic drugs. Research has found that up to 60% of the time doctors prescribe antibiotics they do so incorrectly, whether in situations when they are not needed or in incorrect doses (CDC, 2013; Luty et al., 2014; Lushniak, 2104). Incorrect dosages, especially when suboptimal, are an especially dangerous phenomenon. Sub-therapeutic doses of antibiotics have been found to promote antibiotic resistance by stimulating genetic alterations in bacterial colonies through changes in gene expression, horizontal gene transfer and mutagenesis (Viswanatahn, 2013).

While overuse and inappropriate prescription in medicine have contributed to the antibiotic-resistance crisis, the issue is especially amplified by the extensive use of antibiotics in the agricultural industry. Antibiotics have long been used by farmers to treat both their crops and livestock. Antibiotic treatment increases the overall health of the stock and crops and therefore the overall agricultural yield increases (Michael et al., 2014; Ventola, 2015). In total 15 million kilograms of antibiotics are used yearly in the United States, 80% in the agricultural sector (Bartlett et al., 2013' Spellberg and Gilbert, 2014; Ventola, 2015). Recent research has shown that overuse of antibiotics in livestock can directly harm the humans consuming the meat of that livestock. First, antibiotic usage in the livestock kills non-resistant bacterial strains and allows the resistant bacteria to thrive given the wealth of resources available after the non-resistant bacteria dies. Then, these antibiotic-resistant

bacteria are transferred to the humans who consume the meat of the livestock treated with excessive antibiotics. These antibiotic-resistant bacteria can than then cause serious infections in humans and be transmitted to other individuals from the initial host (CDC, 2013; Ventola, 2015).

The Current State of Antibiotic Drug Development

It would seem logical that, given the immense importance of antibiotics in medicine and the continually worsening antibiotic resistance crisis, the investment in antibiotics research would currently be at a high point but this is simply not the case. Instead, the discovery of new antibiotics has essentially stalled and the financial investment in antibiotic discovery is shockingly low. Less than a decade ago researchers noted that there were currently no antibiotics in advanced development that successfully targeted bacteria already resistant to currently available antibiotics (Boucher et al., 2009). Two studies both note that all but five of the top 50 pharmaceutical companies have stopped funding antibiotics research entirely (Boucher, 2009; O'Neill, 2016). Of the largest 18 pharmaceutical companies, 15 had abandoned the antibiotic field by 2015 (Ventola, 2015). Overall, the researchers noted a 75% decrease in the antibiotics approved by the FDA from the period of 1983-2007 (Boucher, et al., 2009). They estimated the number of new FDA approved antibiotics will continue to drop as there has been a similar decrease in the number of registered early clinical trials for new antibiotics (Boucher, et al., 2009). Two recent studies found that only 40 antibiotics were currently in development and only 14 of those drugs were at or past phase 3 trials (Pew, 2014; Lushniak, 2014). Dr. Lynn Silver, a leading expert on the antibiotic resistance crisis, exclaimed that we have suffered a "discovery void" of over 30 years where efforts to

research novel antibiotic classes have largely failed, “If you look at when things were discovered, we stopped discovering novel antibiotics in 1987” (Chemical Sciences Roundtable, 2014: 7).

A significant cause for this precipitous drop in the discovery and success of novel antibiotics is financially motivated. Since Fleming and Waksman discovered the earliest antibiotics, a vast majority of antibiotic discovery has occurred due to the effort of large pharmaceutical companies. However, over the past thirty or so years multiple factors have combined to de-incentivize pharmaceutical research into novel antibiotic drugs. First, traditional approaches to discover new antibiotics have been largely unsuccessful over the past few decades and novel approaches have proved costly and time consuming (Davies, 2006; Ventola, 2015). Also, stringent FDA clinical requirements oftentimes will take over ten years to approve a drug and therefore by the time of approval the 20-year patent for the drug is already halfway expired (Davies, 2006). Finally, the short and limited nature of antibiotic treatments, along with the relatively low cost of antibiotics in the market, result in antibiotics being a low profit drug (Davies, 2006; Ventola, 2015). Newer antibiotics are priced at a maximum of $1,000 to $3,000 per course, compared to newer chemotherapy drugs which can cost tens of thousands of dollars per course (Ventola, 2015). Also, drugs used to treat chronic disorders have a significantly longer course of treatment than antibiotics. Researcher Martin L. Katz notes that “whereas one patient might require a full year of therapy for a chronically administered drug…most antibiotic drugs are administered for about a week, and therefore, it would take 52 patients to achieve a similar financial return” (Katz et, 2006: 1530). Overall, a study by the London School of Economics found that a new antibiotic has a net present value of $-50 million, compared to a net present value

of +$1 billion for a new musculoskeletal drug (Ventola, 2015; Bartlett et al., 2013). The cost of research, development, production and approval often far surpasses the financial returns a pharmaceutical company will receive once the drug hits the market. All of this combines to form an environment where investment in antibiotic development and production is an unwise financial decision for pharmaceutical companies.

Identifying Tryptophan Synthase as a Potential Antibiotic Target

The research in this paper was motivated by the immense need for new antibiotics and the relative lack of antibiotic research and development in a time of dire need. Following extensive research, the enzyme tryptophan synthase was identified as a potential antibiotic target. Tryptophan synthase is an essential enzyme in bacteria, yeasts, molds and plants that catalyzes the formation of L-tryptophan from indole glycerol phosphate and L-serine (Miles, 2009). Notably, although tryptophan synthase is necessary for the survival of bacteria it is not

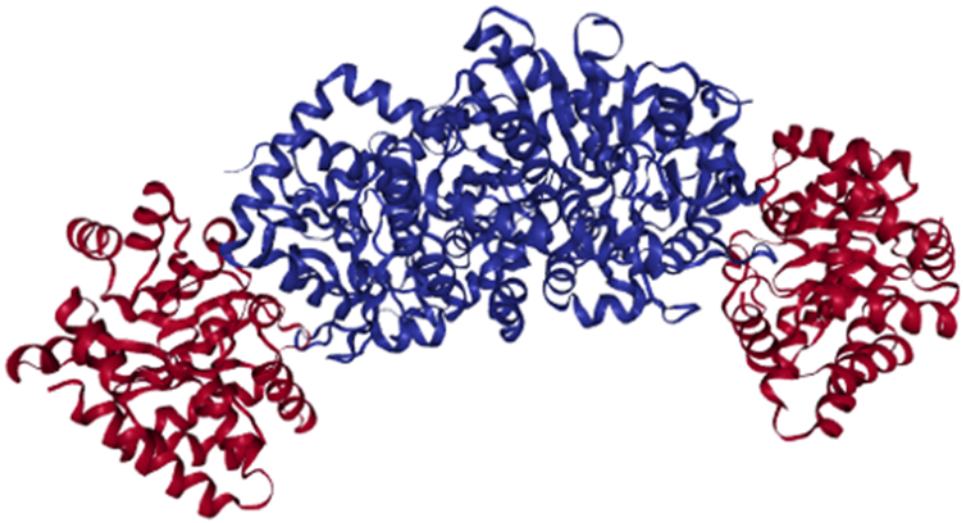

Fig. 1. The crystal secondary structure of wild type *Salmonella typhimurium* tryptophan synthase colored according to the subunits. Red = α-subunits, blue = β_2 dimer (Schneider et al., 1998).

present in humans and other animals. Tryptophan synthase is composed of two α-subunits and one β_2-dimer that combine to form a linear αββα complex (Miles, 2009; Fatimi et al., 2009). The α subunit first catalyzes the reversible lyase of indoleglycerol phosphate (IGP) to an indole group (IND) and glyceraldehyde 3-phosphate (GAP) (Lane and Kirschner, 1991). The indole then travels through a 25 Å long hydrophobic tunnel that allows for direct substrate channeling from the α-active site to the β-active site (Fatimi et al., 2009; Barends et al., 2008). The β_2-dimer then catalyzes the irreversible synthesis of L-tryptophan from IND and L-serine (Lane and Kirschner, 1991). The secondary structure of tryptophan synthase can be seen in the image above (Schneider at al., 1998).

The tryptophan produced from tryptophan synthase is essential for bacterial survival. While tryptophan is an essential amino acid that humans and other animals must consume through their diet, enzymatic production is the primary source of tryptophan in bacteria (Yanofsky, 2007). Tryptophan is an amino acid required for proper protein biosynthesis and many products of tryptophan, such as indole, are biologically essential compounds for bacteria (Yanofsky, 2007). Multiple studies have found that in *E.coli* the indole generated through tryptophan metabolism is involved in both cell-to-cell communication through quorum sensing and biofilm formation (Yanofsky, 2007; Wang et al., 2001; Ren et al., 2004; Winzer et al., 2002). Therefore, a significant lack of tryptophan should be fatal to bacterial cells.

Since tryptophan is biologically essential for bacteria, the inhibition of bacterial enzymes involved in tryptophan biosynthesis have been suggested as potential drug targets (Dias et al., 2006; Chaudhary and Roos, 2005). Notably, all organisms that synthesize tryptophan endogenously do so by a single route and therefore inhibition of one enzyme in

the tryptophan biosynthesis pathway should significantly reduce intracellular tryptophan levels in bacteria (Dias et al., 2006). Past research briefly discussed the inhibition of tryptophan biosynthesis as a potential source for novel antibiotic enzymatic targets when discussing the genomic similarities between bacterial and protozoan tryptophan synthase (Chaudhary and Roos 2005). Later research specifically recommended tryptophan synthase as the most promising enzyme of the tryptophan biosynthesis pathway for the enzymatic target of potential antibiotics (Dias et al., 2006). These researchers noted that tryptophan synthase is the most extensively studied of the enzymes in the tryptophan synthesis pathway (Dias et al., 2006). In fact, earlier research observed that phosphonated inhibitors of tryptophan synthase are already being produced for their antimicrobial and herbicidal potential (Finn et al., 1999). Inhibitors of tryptophan synthase have already been found to be potentially successful broad-spectrum antibiotics (Faulkner et al., 2016).

Furthermore, previous research found that indole produced through tryptophan metabolism is directly involved in the expression of *E. coli* multidrug exporter genes (Hirakawa et al., 2005). These exporter genes are a common method of bacterial antibiotic multidrug resistance. The researchers found that in normal conditions these multidrug exporter genes are poorly expressed, however increased intracellular indole concentrations led to the overexpression of the exporter genes (Hirakawa et al, 2005). The amplified expression of the drug exporter genes, upregulated by the presence of indole, conferred multidrug resistance to *E. coli* colonies (Hirakawa et al., 2005). Inhibition of tryptophan synthase and the obstruction of tryptophan biosynthesis may therefore potentially prevent the multidrug resistance of *E. coli* and other bacteria with multidrug exporter genes.

Overall, the research suggests that tryptophan synthase is a promising target for antibiotic enzymatic inhibition. Not only may inhibition of tryptophan synthesis succeed as a broad-spectrum antibiotic target, but it also may act as an adjuvant, decreasing the potential for the development of resistance to other antibiotics. In fact, a recent study found that 5-alkyloxytryptamines, tryptamine analogs from same base molecule as was utilized in this study, were successful both as broad-spectrum antibiotics and antibiotic adjuvants that potentiated the activity of current clinical antibiotics in Gram-positive and Gram-negative bacteria species (Faulkner et al., 2016).

Tryptamine Analogs as Novel Antibiotics

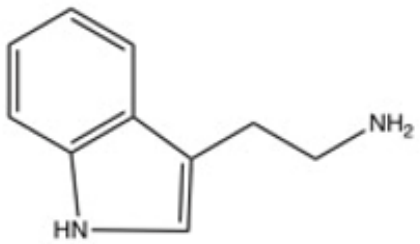

Fig. 2. Molecular structure of tryptamine.

Tryptamine has long been known as an inhibitor of bacterial tryptophan synthase. Research in the 1960's discovered that tryptamine significantly decreased tryptophan synthase activity; at 500 μg/ml tryptamine completely inhibited tryptophan synthase activity in whole cells (Frendlich and Lichstein, 1961). Furthermore, these same researchers importantly found that tryptamine not only inhibited tryptophan synthase activity but also repressed the growth of *E.coli* cultures (Freundlich and Lichstein, 1961). Although these

study results showed that tryptamine and related molecules may be successful as potential antibiotics, little published research can be found on the topic in the preceding three decades.

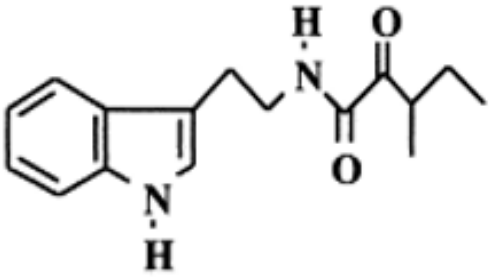

Fig. 3. Molecular structure of nematophin, a tryptamine analog antibiotic isolated from the soil microbe *Xenorhabdus nematophilus* (Jianxiong et al., 1997).

Over thirty years later researchers published a paper that identified Nematophin, a novel antibiotic from the soil microbe *Xenorhabdus nematophilus* (Jianxiong et al., 1997). Nematophin was identified as a tryptamine analog and was found to be a highly active antibiotic against both wild-type and drug-resistant strains of *S.aureus* (Juanxiong et al., 1997). In the same paper these researchers also identified ten other Nematophin analogs, all of which were tryptamine analogs and displayed antibiotic properties, albeit weaker than the original nematophin (Jianxiong et al., 1997).

Fifteen years later, different researchers published a paper that analyzed and identified a tryptamine-related antibiotic produced from the soil bacterium *Intrasporangium* N8 (Okudoh and Wallis, 2012). An earlier study by these same scholars found that the substance produced by the *Intrasporangium* was an antibiotic with significant antibacterial activity against both Gram-positive and Gram-negative bacteria (Okudoh and Wallis, 2007). Through the method of gas chromatography-mass spectroscopy (GC-MS) these investigators identified that one of four components in the antibiotic substance discovered in 2007 was N-

acetyltryptamine, a tryptamine analog (Okudoh and Wallis, 2012). Importantly, the researchers also noted that further research identified N-acetyltryptamine as the component responsible for the observed antibiotic qualities of the substance and that "the antibiotic produced by *Intrasporangium* strain N8 has the same or a similar mechanism of action to tryptamine" (Okudoh and Wallis, 2012: 737).

A recent study continued this research into tryptamine analogs as potential antibiotic agents (Faulkner et al., 2016). They advanced earlier research by displaying that not only may tryptamine analogs be potentially successful broad-spectrum antibiotics, but they may also have applications as antibiotic adjuvants. Overall, the prior research has displayed serious potential for tryptamine analogs as successful broad-spectrum antibiotics and antibiotic adjuvants. However, the noticeable lack of tryptamine analogs currently in the pipeline for eventual FDA approval and production displays a need for the development of novel tryptamine analogs with antibiotic properties. The present work focuses on the development of novel tryptamine analogs and their potential use as novel antibiotics targeting the inhibition of the tryptophan synthase enzyme.

Materials and Methods

Nomenclature and Identification System of the Tryptamine Analogs

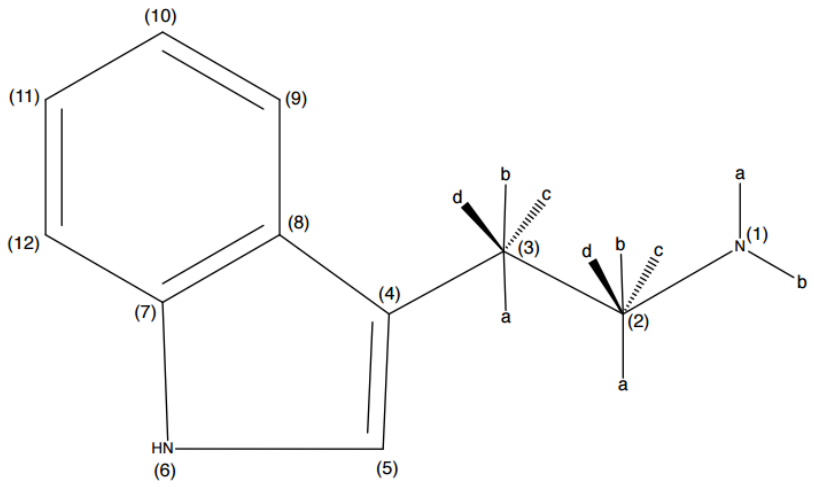

Fig. 4. Labeled molecular diagram of the base tryptamine molecule whereas all base atoms are labeled 1-12 and multiple possible stereochemical arrangements at a single base atom are labeled a-d.

In order to clearly label and identify the tryptamine analogs we developed a nomenclature system of the original tryptamine molecule which labeled all of the non-hydrogen atoms with the numbers 1-12. If the base atoms had multiple possible stereochemical orientations these different orientations were labeled a – b or a – d depending on the number available orientations. For the illustration of this nomenclature system we used ChemDraw Professional 16.0 to draw a diagram of the labeled atoms (PerkinElmer, 2017). The labeled molecular diagram of the base tryptamine molecule is shown in Fig. 4.

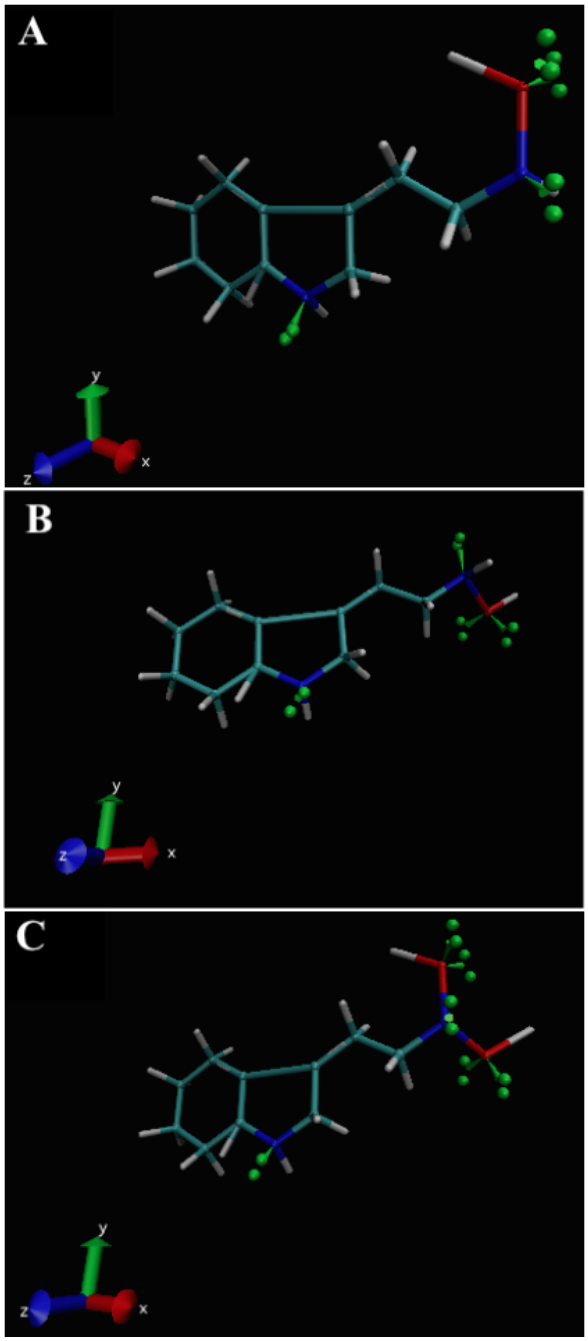

Fig. 5. Possible stereochemical orientations of tryptamine analogs with functional group addition at base atom 1 (White = hydrogen, Teal = Carbon, Red = Oxygen, Blue = Nitrogen). **(A)** 1a; **(B)** 1b; **(C)** 1(2).

Molecular Modeling of the Tryptamine Analogs

For modeling and design of the tryptamine analogs we used Visual Molecular Dynamics (VMD), a computer program for automated modeling and modification of molecules (Humphrey et al., 1996). The crystal structure data of wild-type tryptamine was used as the starting model (Nowell et al., 2002). The VMD Molecule Molefacture program was then used to replace specific hydrogens on the wild-type tryptamine molecule with one of four functional groups: amine, hydroxyl, methyl or carbonyl. In the first round of design each tryptamine analog only contained one of four functional groups attached to a numbered base atom in a specific stereochemical alignment. In situations where a single base atom had multiple available hydrogens to remove and replace, two of the same functional group were attached to that same base atom. The

possible stereochemical orientations of additions to the nitrogen at base atom 1 are seen in Fig. 5. The stereochemical alignments of the groups attached to the nitrogen were identical

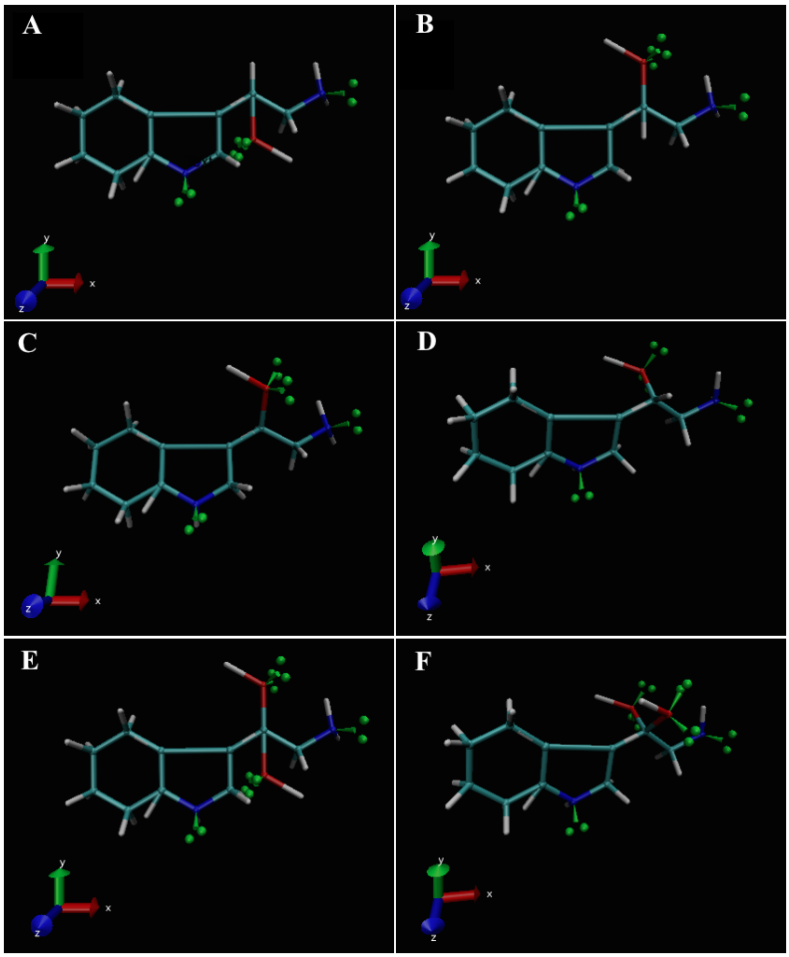

Fig. 6. Possible stereochemical orientations of tryptamine analogs with a functional group addition at base atom 3 (White = hydrogen, Teal = Carbon, Red = Oxygen, Blue = Nitrogen). **(A)** 3a; **(B)** 3b; **(C)** 3c; **(D)** 3d; **(E)** 3(2)a; **(F)** 3(2)b.

across the other three functional groups. When performing modification on the carbons at base atoms 2 and 3, there were four different stereochemical orientations for additions of a single functional group and two unique stereochemical orientations for addition of two of the same functional group. The possible stereochemical alignments of functional groups attached at carbon 3 are seen in Fig. 6. These stereochemical orientations are consistent across all

other functional groups and at both of the carbons at positions two and three. Finally, additions at the carbons along the indole ring, at locations 5 and 9-12 only occurred in a single stereochemical orientation. A second round of tryptamine analogs with multiple functional groups attached at multiple different numbered base atoms were created soon after the analysis of the initial binding affinity data and followed the same identification system. In total, seventy-two unique tryptamine analogs were created through this process.

Binding Affinity Analysis of Tryptophan Synthase Subunits-Tryptamine Analogs Complexes

For the binding affinity analyses of the complexes between the tryptophan synthase subunits and tryptamine analogs we used PyRx, a computer program for screening libraries of compounds against potential drug targets (Dallakyn and Olson, 2015). Three separate crystal structures of tryptophan synthase were utilized in this study: the structures of the α-subunit and the β_2-dimer of *E. coli* tryptophan synthase and the β_2-dimer of *M.tuberculosis* (Nishio et al., 2015; Nishio et al., 2016; Kachalova et al.). For the three enzyme crystal structures we used the PDB access codes: 1V7Y, 2DH5 and 2O2E respectively. In order to simulate the protein-ligand complexes between the tryptophan synthase subunits and tryptamine analogs the crystal structures of the *E. coli* α-subunit and the *E. coli* and *M.tuberculosis* β_2-dimers were loaded directly into the PyRx program. First, before the protein-ligand complexes were formed, all 72 of the tryptamine analogs and wild-type tryptamine were energetically minimized using the PyRx program. After the minimization process was completed we used the PyRx software to simulate the formation of the protein-ligand complexes between the tryptophan synthase subunits and the tryptamine analogs. For

all three tryptophan synthase subunits the entire macromolecule was selected as a potential binding region for the tryptamine analogs. All potential binding interactions between the tryptophan synthase subunits and each individual tryptamine analog, including wild-type tryptamine, were then simulated. Estimates of the nine strongest binding affinities (kcal/mol) for each protein-ligand complex were provided by PyRx. The binding affinities were presented as negative values and the more negative the kcal/mol value the stronger the binding affinity between the protein-ligand complex. An increase in binding affinity of – 1 kcal/mol correlated to an approximately ten-fold increase in the binding affinity between the protein and the ligand. Between the 72 tryptamine analogs, wild-type tryptamine and three tryptophan synthase subunits, 219 unique protein-ligand complexes were modeled and 1,971 binding affinity measurements were taken. The strongest binding affinity for each protein-ligand complex was then separated and we graphed the results using the computer program R and the package ggplot2 (R Core Team, 2016; Wickham, 2009).

Binding Affinity Analysis of Human MAOB-Tryptamine Analogs Complexes

For the binding affinity analyses of the complexes between human MAOB and the tryptamine analogs we used PyRx again (Dallakyn and Olson, 2015). The crystal structure of human MAOB, PDB access code 1GOS, was used as the enzymatic model for MAOB (Binda et al., 2001). The human MAOB crystal structure and the already energetically minimized wild-type tryptamine and tryptamine analogs were loaded directly into PyRx. Then, we used the PyRx software to simulate the formation of protein-ligand complexes between the MAOB enzyme and the tryptamine molecules. Again, the entire MAOB enzyme was selected as a potential binding region for the tryptamine analogs. Estimates of the nine

strongest binding affinities (kcal/mol) for the MAOB-wild-type tryptamine complex and each of the 72 MAOB-tryptamine analog complexes were provided by PyRx. The strongest binding affinity value for each complex was then separated and we again graphed the results using the computer program R and the package ggplot 2 (R Core Team, 2016; Wickham, 2009).

Molecular Modeling of Complexes Between Tryptophan Synthase Subunits or Human MAOB and Identified Tryptamine Analogs Complexes

For the modeling of the protein-ligand complexes between the tryptophan synthase subunits or human MAOB and the ten identified tryptamine analogs we used the program PyMOL (Schrödinger). Following the binding affinity analyses the protein-ligand complexes formed in PyRx were exported to PyMOL. Within PyMOL the location the ten tryptamine analogs bound to the surface of the tryptophan synthase subunits or human MAOB with the highest binding affinity was visualized and analyzed. The number of hydrogen bonds that each tryptamine analog formed with the enzyme of question was determined. Pictures of the binding sites and hydrogen bond formations were taken for each protein-ligand complex that the ten identified tryptamine analogs formed. This process was then repeated with the complexes formed by wild-type tryptamine and the tryptophan synthase subunits and human MAOB. The binding regions and hydrogen bond formation of the tryptamine analogs were then compared to wild-type tryptamine for each of the four enzymatic targets.

Results

Binding Affinity Analysis of Tryptophan Synthase Subunits-Tryptamine Analogs Complexes

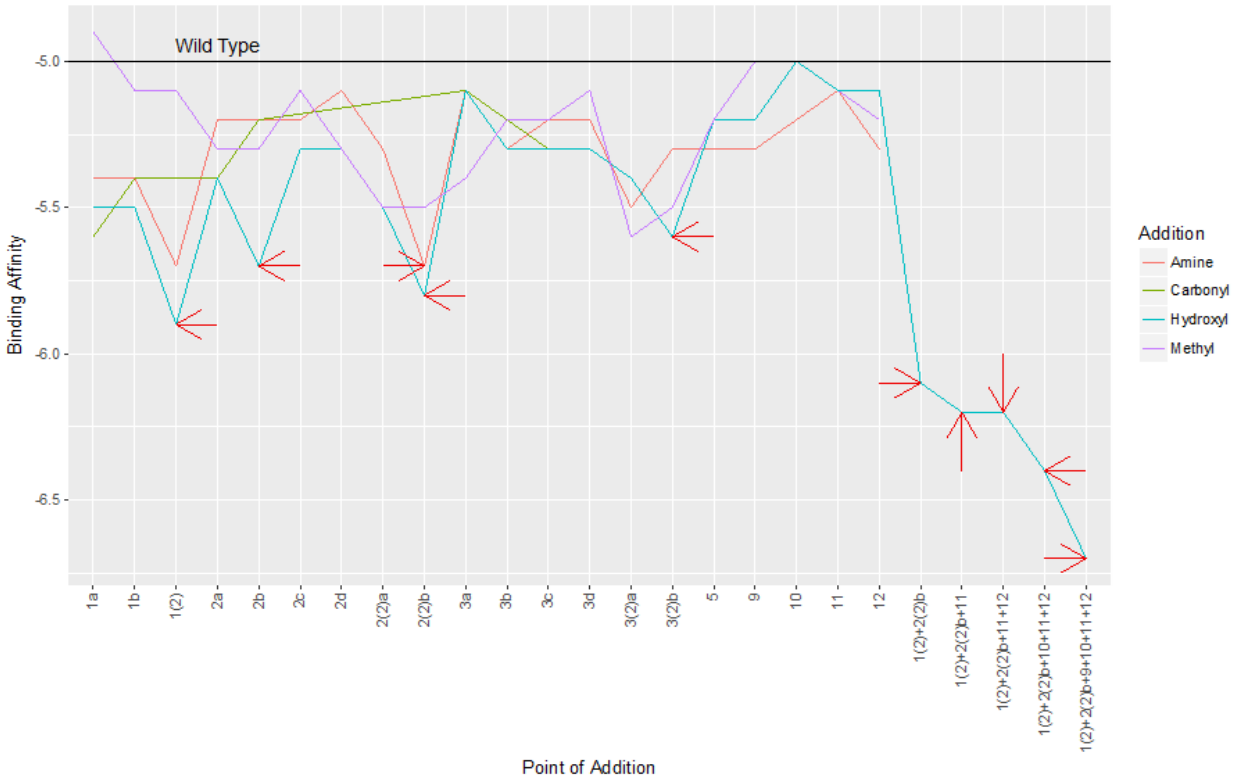

Fig. 7. Comparison between the binding affinities (kcal/mol) of the tryptamine analogs and the *E. coli* tryptophan synthase α-subunit to the binding affinity of wild-type tryptamine and the same subunit. The red arrows identify the ten tryptamine analogs identified for further research.

Line plots were generated containing the highest binding affinity value of each tryptamine analog to the *E. coli* α-subunit. These values were compared to the binding affinity of wild-type tryptamine to the *E. coli* α-subunit. The plot of the binding affinities of wild-type tryptamine and the tryptamine analogs to the *E. coli* α-subunit is presented in Fig. 7. Almost every tryptamine analog had a higher binding affinity to the *E. coli* α-subunit than

wild-type tryptamine, however ten tryptamine analogs displayed a noticeably larger increase in binding affinity than the others.

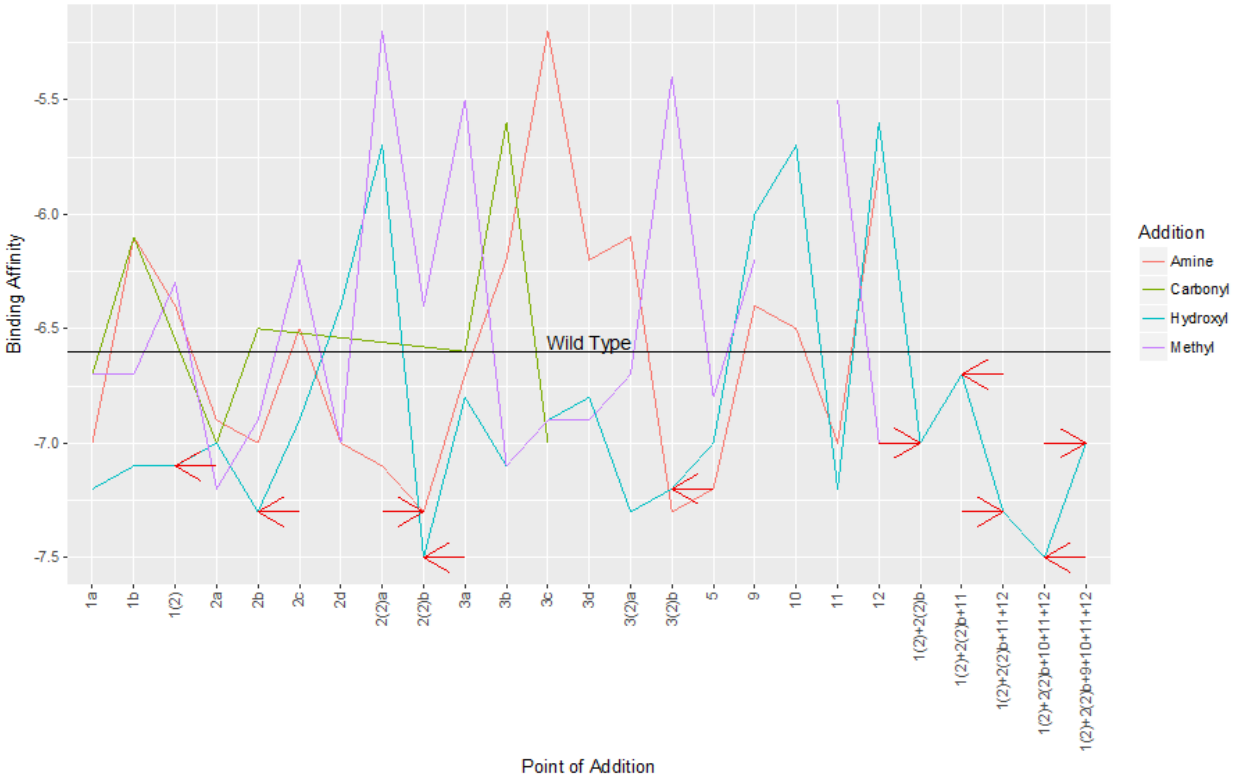

Fig. 8. Comparison between the binding affinities (kcal/mol) of the tryptamine analogs and the *E. coli* tryptophan synthase β_2-dimer to the binding affinity of wild-type tryptamine and the same subunit. The red arrows identify the ten tryptamine analogs identified for further research.

Line plots were then produced containing the highest binding affinity value of each tryptamine analog to the *E. coli* β_2 dimer. The binding affinities of the tryptamine analogs were compared to the binding affinity of wild-type tryptamine to the *E. coli* β_2 dimer. The plot of the binding affinities of wild-type tryptamine and the tryptamine analogs to the *E. coli* β_2 dimer is presented in Fig. 8. The binding affinity of wild-type tryptamine to the *E. coli* β_2 dimer was significantly stronger than the wild-type binding affinity to the *E. coli* α-subunit and therefore many of the tryptamine analogs weakened the binding affinity to the *E. coli* β_2

dimer. However, once again there were several tryptamine analogs that displayed an increased binding affinity to the *E. coli* β_2 dimer. The ten analogs identified in the past section all exhibited a greater binding affinity to the *E. coli* β_2 dimer than wild-type tryptamine, although in same cases other analogs caused an even greater increase in binding affinity.

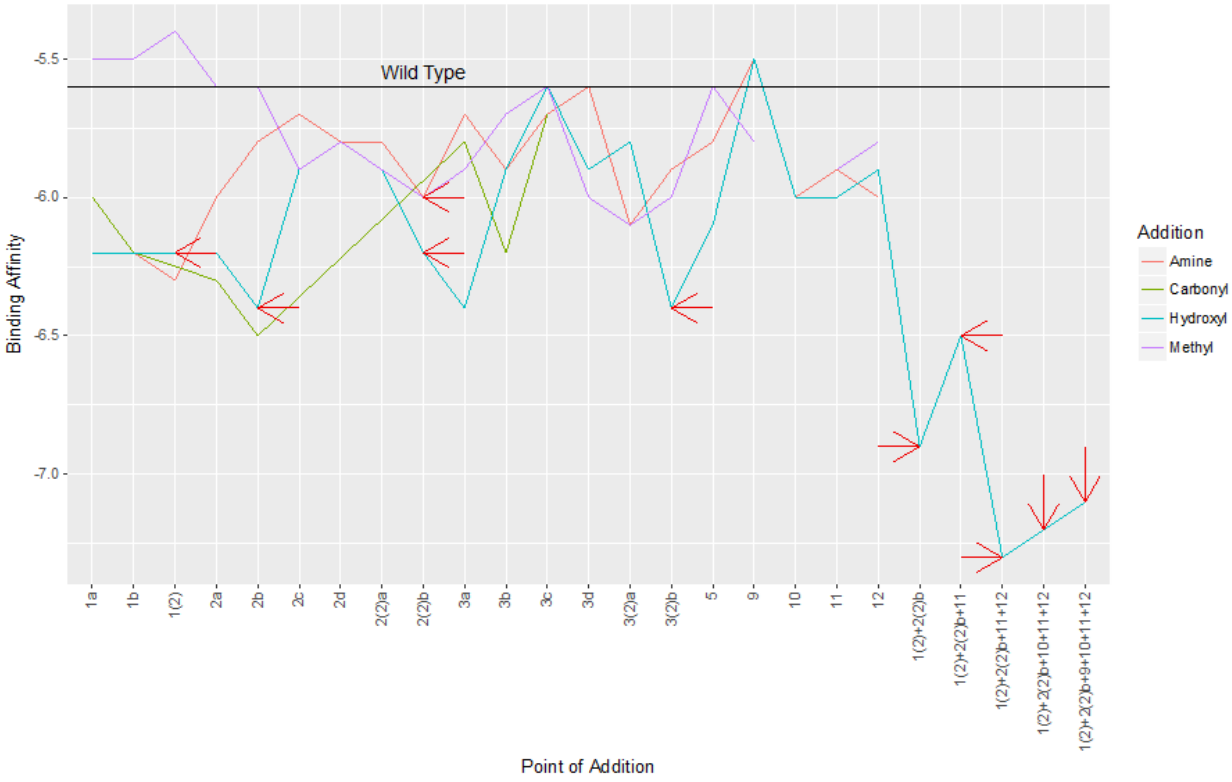

Fig. 9. Comparison between the binding affinities (kcal/mol) of the tryptamine analogs and the *M. tuberculosis* tryptophan synthase β_2-dimer to the binding affinity of wild-type tryptamine and the same subunit. The red arrows identify the ten tryptamine analogs identified for further research.

A third line plot was created containing the highest binding affinity value of each tryptamine analog to the *M. tuberculosis* β_2 dimer. As before, the binding affinities of the tryptamine analogs were compared to the binding affinity of wild-type tryptamine to the *M. tuberculosis* β_2 dimer. This plot of the binding affinities of wild-type tryptamine and the tryptamine analogs to the *M. tuberculosis* β_2 dimer is presented in Fig. 9. The clear majority

of tryptamine analogs displayed a significantly stronger binding affinity to the *M. tuberculosis* β_2 dimer than wild-type tryptamine. Again, although a few other analogs demonstrated a greater increase in their binding affinities to the *M. tuberculosis* β_2 dimer, the ten analogs identified earlier showed a significant increase in binding affinities to the *M. tuberculosis* β_2 dimer.

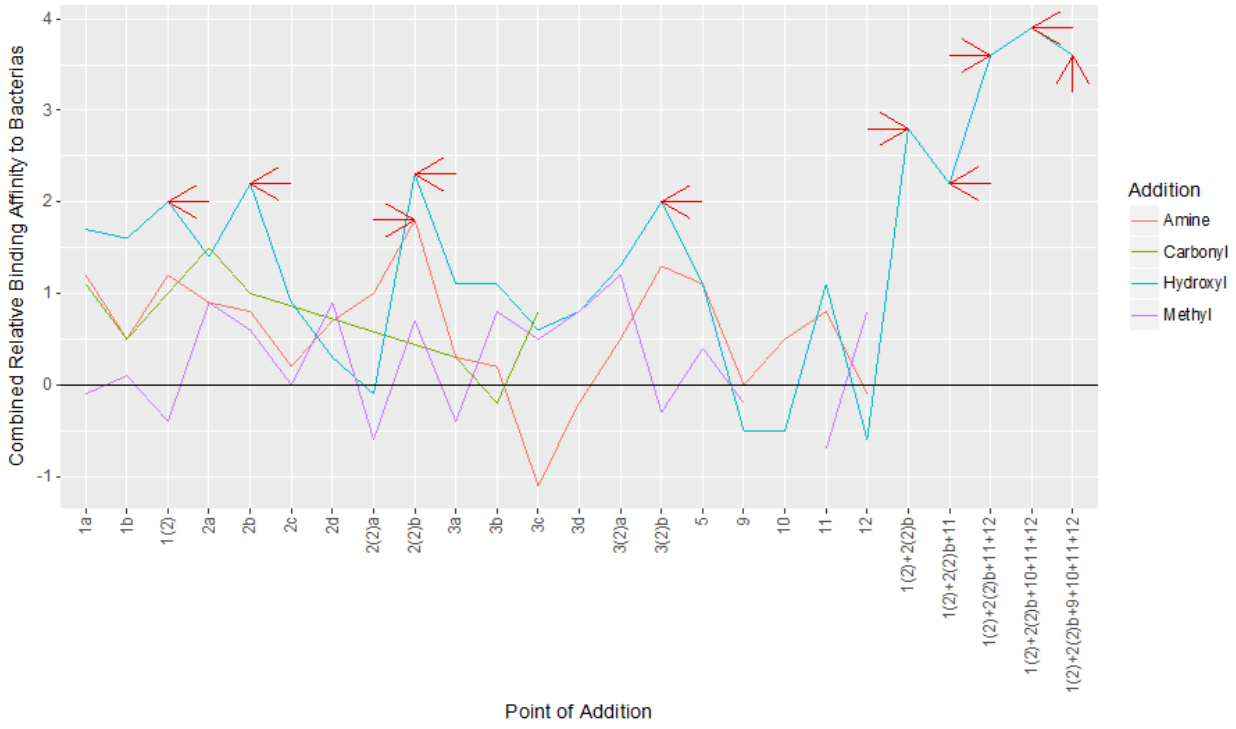

Fig. 10. Summation of the relative differences between the binding affinities (kcal/mol) of the tryptamine analogs and wild-type tryptamine to the three tryptophan synthase subunits used in this paper. The red arrows identify the ten tryptamine analogs identified for further research.

To quantify the potential of the tryptamine analogs as broad-spectrum antibiotic agents we calculated each analog's relative change from wild-type tryptamine in binding affinity to each of the three tryptophan synthase subunits. These three values were then added together to ascertain each tryptamine analogs' combined increase in relative binding affinity to the tryptophan synthase subunits. The plot of each analog's combined relative change from

wild-type tryptamine in binding affinity to the three bacterial tryptophan synthase subunits is presented in Fig.10. Several tryptamine analogs displayed a decrease from wild-type tryptamine in the combined binding affinity to the three tryptophan synthase subunits. However, a definite majority demonstrated an increase in the combined binding affinity to the tryptophan synthase subunits when compared to wild-type tryptamine. Noticeably, the ten tryptamine analogs identified in the earlier sections displayed the largest increases in the combined relative binding affinity.

Binding Affinity Analysis of Human MAOB-Tryptamine Analogs Complexes

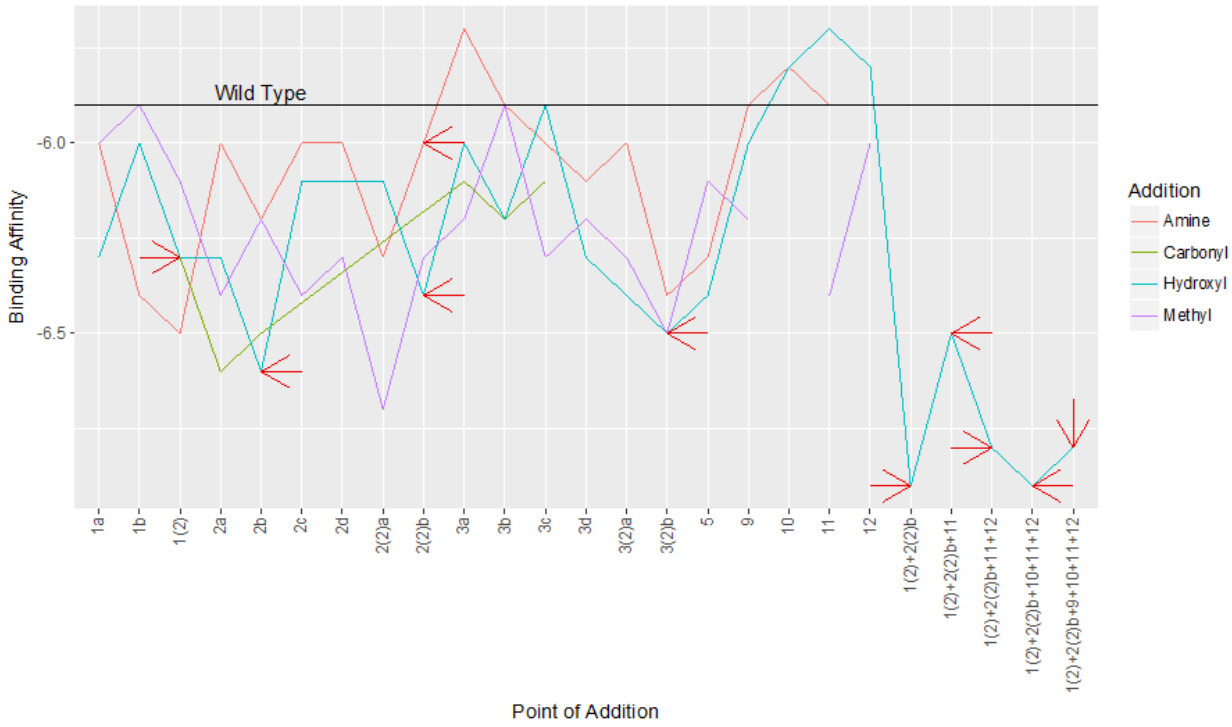

Fig. 11. Comparison between the binding affinities (kcal/mol) of the tryptamine analogs and human MAOB to the binding affinity of wild-type tryptamine to MAOB. The red arrows identify the ten tryptamine analogs identified for further research.

After testing the effect of the tryptamine modifications on the molecules' binding affinities to bacterial tryptophan synthase, we utilized similar methods to test the effects of the same modifications on the tryptamine analogs' binding affinity to human MAOB. Line plots were produced containing the highest binding affinity value of each tryptamine analog to the human MAOB. Then, we compared the binding affinities of the tryptamine analogs to human MAOB and the binding affinity of wild-type tryptamine to human MAOB. The plot of the binding affinities of wild-type tryptamine and the tryptamine analogs to human MAOB is presented in Fig. 11. In an attempt to visualize the differences in binding affinities to human MAOB between the tryptamine analogs and wild-type tryptamine we created a graph of each analog's relative difference from wild-type tryptamine in MAOB binding affinity,

seen in Fig.12. Although a few tryptamine analogs were able to decrease the binding affinity to human MAOB, a significant majority displayed an increased MAOB binding affinity when compared to wild-type tryptamine. Similarly, all ten of the promising tryptamine analogs showed an increased human MAOB binding affinity. However, the one tryptamine analog that involved the addition of an amine group, and not a hydroxyl group, displayed the smallest MAOB binding affinity increase of the ten identified molecules.

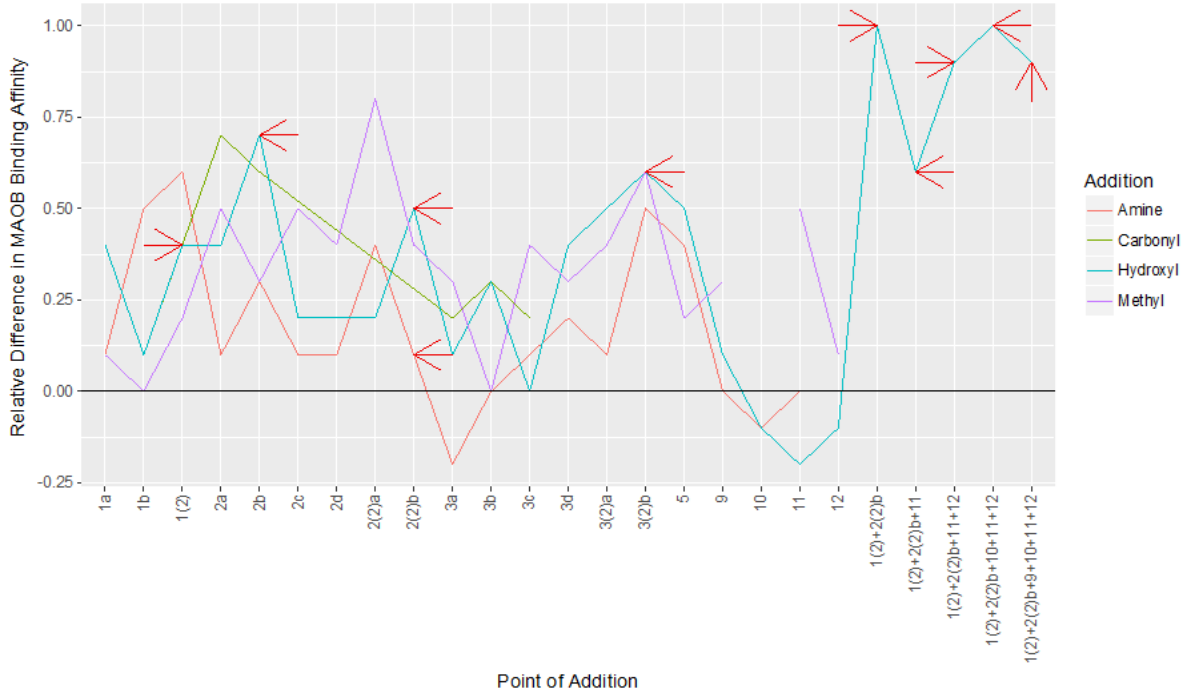

Fig. 12. The relative difference in the binding affinity (kcal/mol) to MAOB of the tryptamine analogs from wild-type tryptamine. The red arrows identify the ten tryptamine analogs identified for further research.

Integrating the Tryptamine Analogs' Binding Affinities to Tryptophan Synthase and MAOB

Finally, we designed an analysis to integrate both the tryptamine analogs' binding affinities to the three tryptophan synthase subunits and the analogs' binding affinities to the human MAOB. To accomplish this, we subtracted the relative difference in binding affinity to MAOB between the tryptamine analogs and wild-type tryptamine from the summated relative difference in binding affinity to the tryptophan synthase subunits between the tryptamine analogs and wild-type tryptamine. The plot of this difference in relative binding affinities is presented in Fig. 14. Overall, a majority of tryptamine analogs had a positive difference in the relative binding affinity change from wild-type between the tryptophan synthase subunits and human MAOB. Significantly, the ten aforementioned tryptamine analogs displayed the highest differences in relative binding affinity.

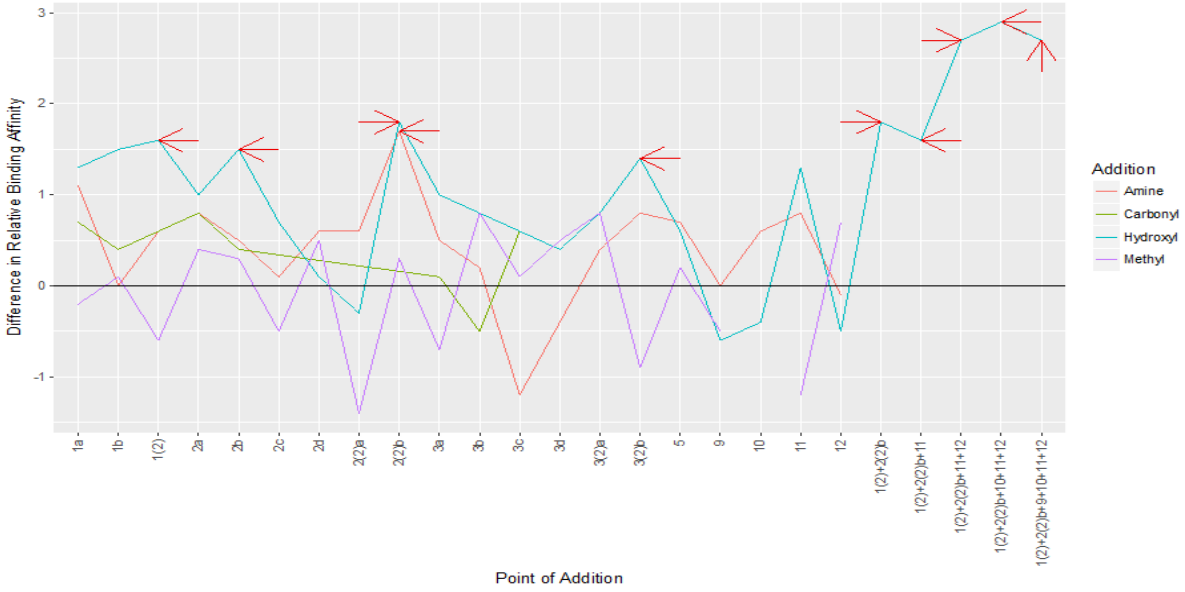

Fig. 13. The difference between the combined increase in relative binding affinity (kcal/mol) of the tryptamine analogs from wild-type tryptamine to the three tryptophan synthase subunits and the relative difference in the binding affinity of the tryptamine analogs from wild-type tryptamine to human MAOB. The red arrows identify the ten tryptamine analogs identified for further research.

Identification of the Tryptamine Analogs Deserving Synthesis and Further Research

Following the extensive binding affinity analyses, we identified ten tryptamine analogs that present the most promise as tryptophan synthase inhibitors. The molecular structure of these ten tryptamine analogs are pictured in Fig. 14. Nine of the most promising tryptamine analogs included additions of one, or multiple, hydroxyl functional groups and the tenth resulted from an addition of two amine groups at the carbon at position two. Of the nine identified tryptamine analogs involving the addition of a hydroxyl group, one had only a single hydroxyl group addition while the other eight resulted from the addition of multiple hydroxyl groups at one, or several different, base atoms.

Several of the tryptamine analogs generated by the VMD program possess a significantly stronger binding affinity than wild-type tryptamine to each of the three tryptophan synthase subunits. Furthermore, when compared with wild-type tryptamine, the ten most promising tryptamine analogs display an increase of 1.8 kcal/mol – 3.9 kcal/mol in combined relative binding affinity across the three tryptophan synthase subunits. The increases in tryptophan synthase subunit binding affinities for 1(2)+(2)b+10+11+12 hydroxyl tryptamine molecule, the analog that exhibited the largest combined increase in relative binding affinity, correlated to an approximately 14 times increase in the binding affinity to E. coli tryptophan synthase α-subunit, a 9 times increase to the E. coli β2-dimer and a 16 times increase to the M. tuberculosis β2-dimer.

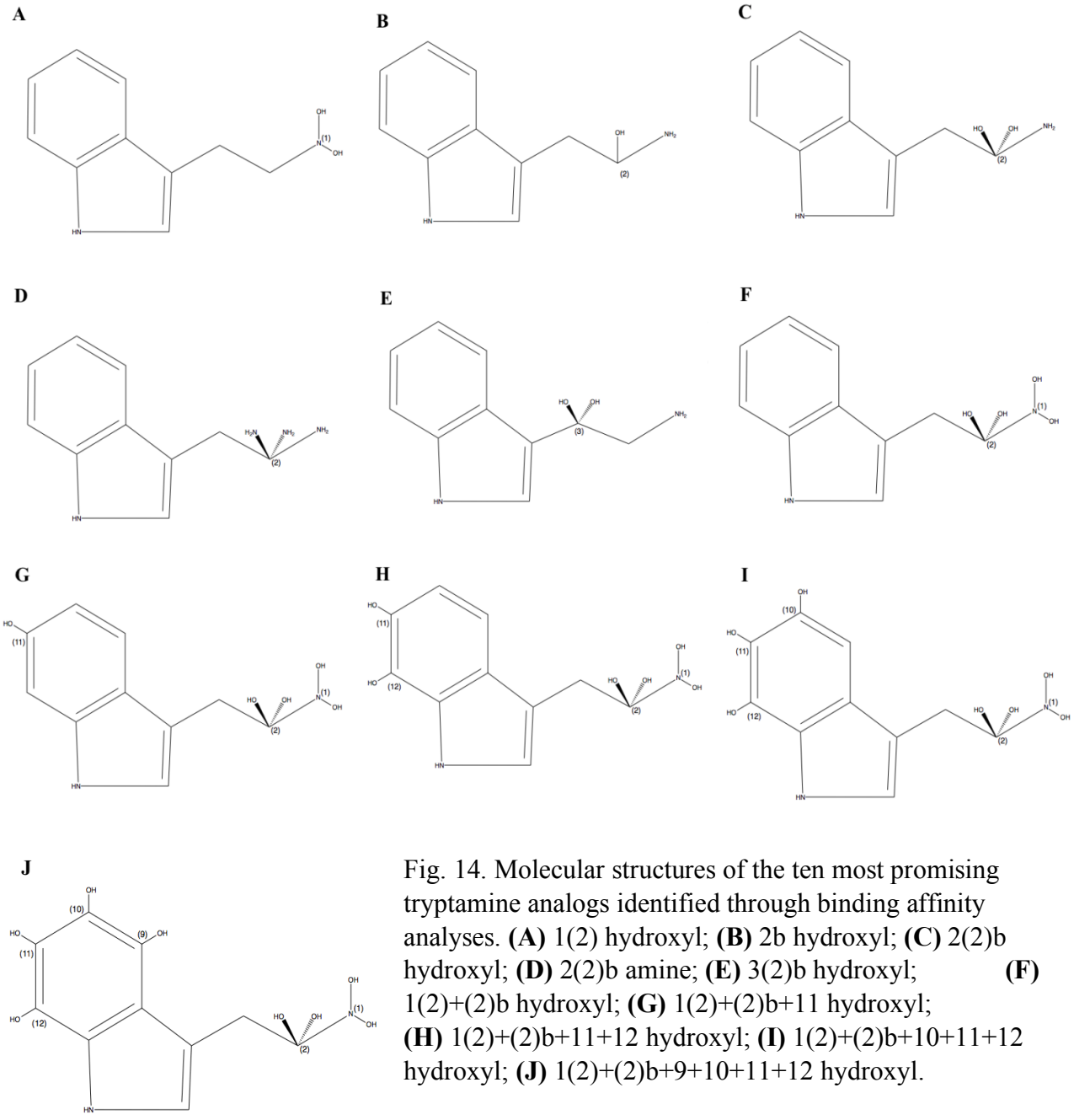

Fig. 14. Molecular structures of the ten most promising tryptamine analogs identified through binding affinity analyses. **(A)** 1(2) hydroxyl; **(B)** 2b hydroxyl; **(C)** 2(2)b hydroxyl; **(D)** 2(2)b amine; **(E)** 3(2)b hydroxyl; **(F)** 1(2)+(2)b hydroxyl; **(G)** 1(2)+(2)b+11 hydroxyl; **(H)** 1(2)+(2)b+11+12 hydroxyl; **(I)** 1(2)+(2)b+10+11+12 hydroxyl; **(J)** 1(2)+(2)b+9+10+11+12 hydroxyl.

Molecular Modeling of Complexes Between Tryptophan Synthase Subunits or Human MAOB and Identified Tryptamine Analogs Complexes

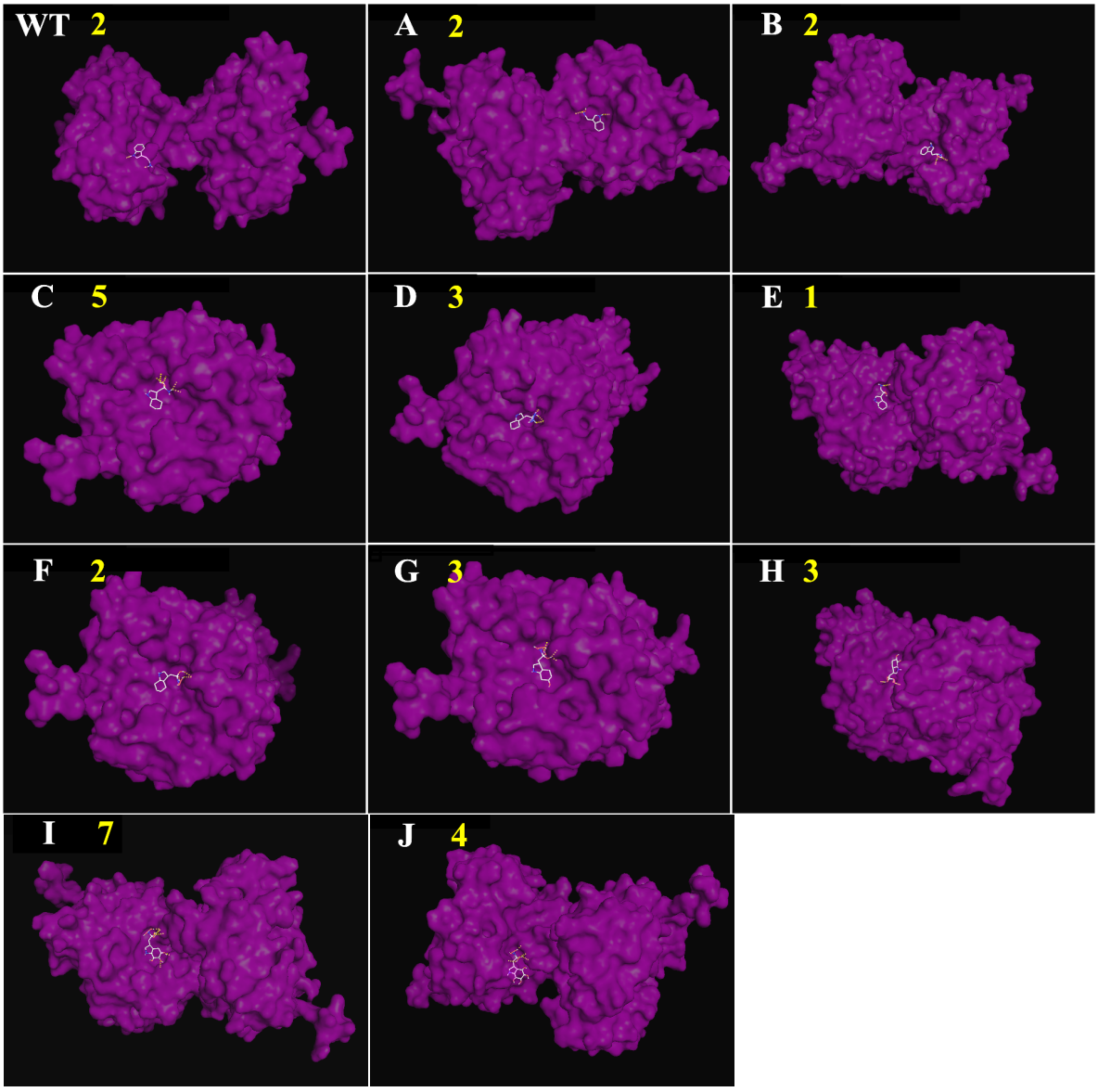

Fig. 15. Molecular models of the strongest binding interactions of the *E.coli* tryptophan synthase α-subunit (purple) and **(WT)** wild-type tryptamine, **(A)** 1(2) hydroxyl, **(B)** 2b hydroxyl, **(C)** 2(2)b hydroxyl, **(D)** 2(2)b amine, **(E)** 3(2)b hydroxyl, **(F)** 1(2)+(2)b hydroxyl, **(G)** 1(2)+(2)b+11 hydroxyl, **(H)** 1(2)+(2)b+11+12 hydroxyl, **(I)** 1(2)+(2)b+10+11+12 hydroxyl and **(J)** 1(2)+(2)b+9+10+11+12 hydroxyl. Yellow numbers represent the number of hydrogen bonds in the binding interaction.

After ten tryptamine analogs were identified through the binding affinity analyses we modeled the strongest binding interaction of these ten tryptamine analogs to the *E. coli* tryptophan synthase α-subunit. Within this model we visualized the number of hydrogen bonds that each tryptamine analog formed with the α-subunit. The pictures of the strongest binding interaction for the ten identified tryptamine analogs, including the hydrogen bonds formed, is presented in Fig. 15. None of the ten identified tryptamine analogs bound with the highest affinity to the same region of the α-subunit as wild-type tryptamine. Furthemore, there was large variance in the the area of strongest binding affinity between the tryptamine analogs. In total we observed five general regions of the α-subunit where one or more of the tryptamine analogs bound with the highest affinity. However, when compared with wild-type tryptamine, a majority of the tryptamine analogs increased the number of hydrogen bonds formed with the α-subunit. Only one analog decreased the number of hydrogen bonds formed with the α-subunit.

We then modeled the strongest binding interaction of the ten identified tryptamine analogs to the *E.coli* tryptophan synthase β_2-dimer. Within this model we visualized the number of hydrogen bonds that each tryptamine analog formed with the β_2-dimer. The pictures of the tryptamine analogs' strongest binding interactions with the *E. coli* β_2-dimer, including the hydrogen bonds formed, is presented in Fig. 16. While there was some variation in the orientation of binding, all ten of the identified tryptamine analogs bound with the highest affinity to the same region of the β_2-dimer as wild-type tryptamine. Also, a majority of the tryptamine analogs increased the number of hydrogen bonds formed with the *E. coli* β_2-dimer. No analogs decreased the number of hydrogen bonds formed with the α-subunit.

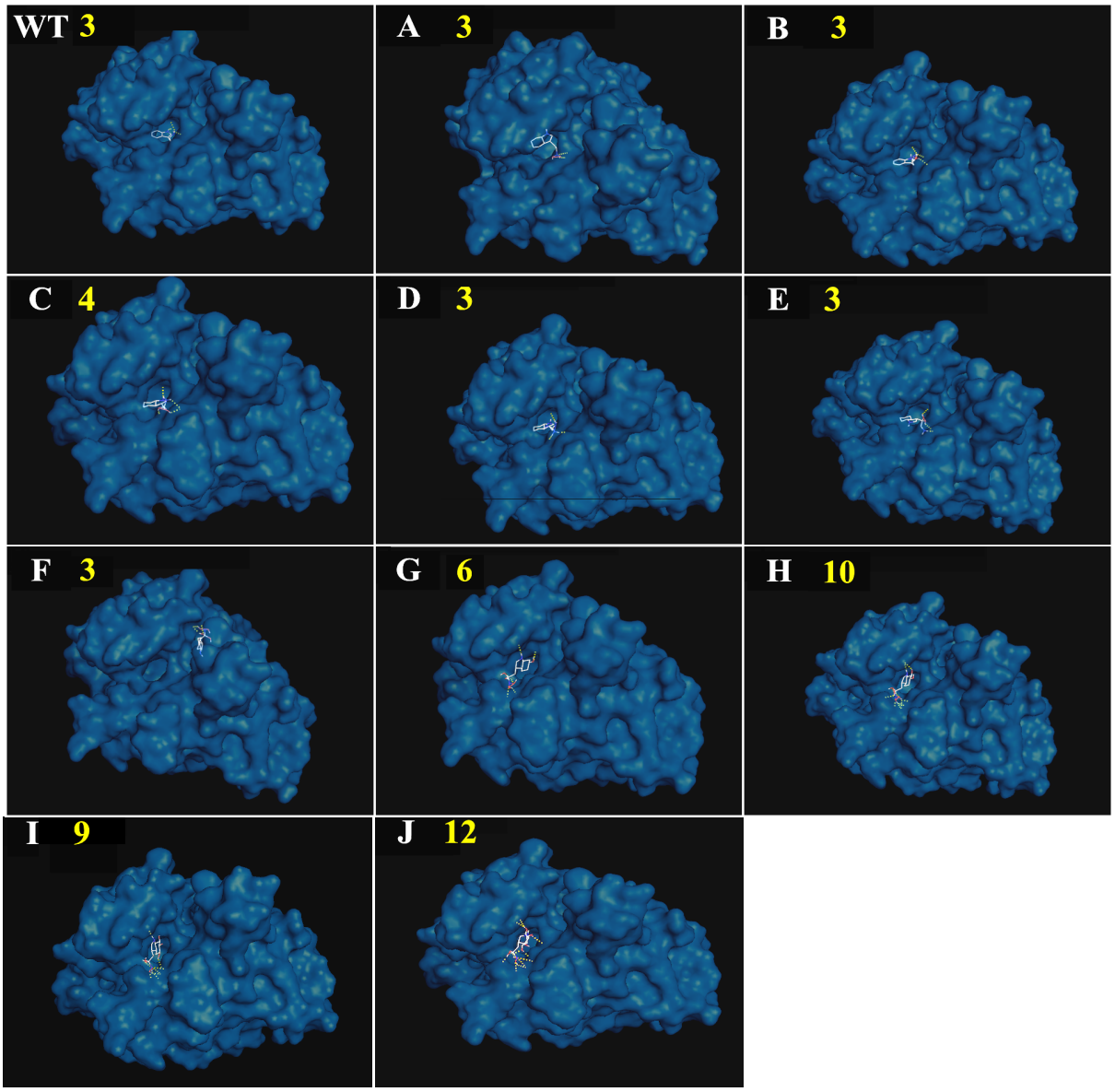

Fig. 16. Molecular models of the strongest binding interactions of the *E.coli* tryptophan synthase β_2-dimer (blue) and **(WT)** wild-type tryptamine, **(A)** 1(2) hydroxyl, **(B)** 2b hydroxyl, **(C)** 2(2)b hydroxyl, **(D)** 2(2)b amine, **(E)** 3(2)b hydroxyl, **(F)** 1(2)+(2)b hydroxyl, **(G)** 1(2)+(2)b+11 hydroxyl, **(H)** 1(2)+(2)b+11+12 hydroxyl, **(I)** 1(2)+(2)b+10+11+12 hydroxyl and **(J)** 1(2)+(2)b+9+10+11+12 hydroxyl. Yellow numbers represent the number of hydrogen bonds in the binding interaction.

Following the modeling of both *E. coli* tryptophan synthase subunits we modeled the strongest binding interaction of the ten identified tryptamine analogs to the *M. tuberculosis* tryptophan synthase β_2-dimer. We also vizualized the number of hydrogen bonds that each tryptamine analog formed with the β_2-dimer. The pictures of the tryptamine analogs'

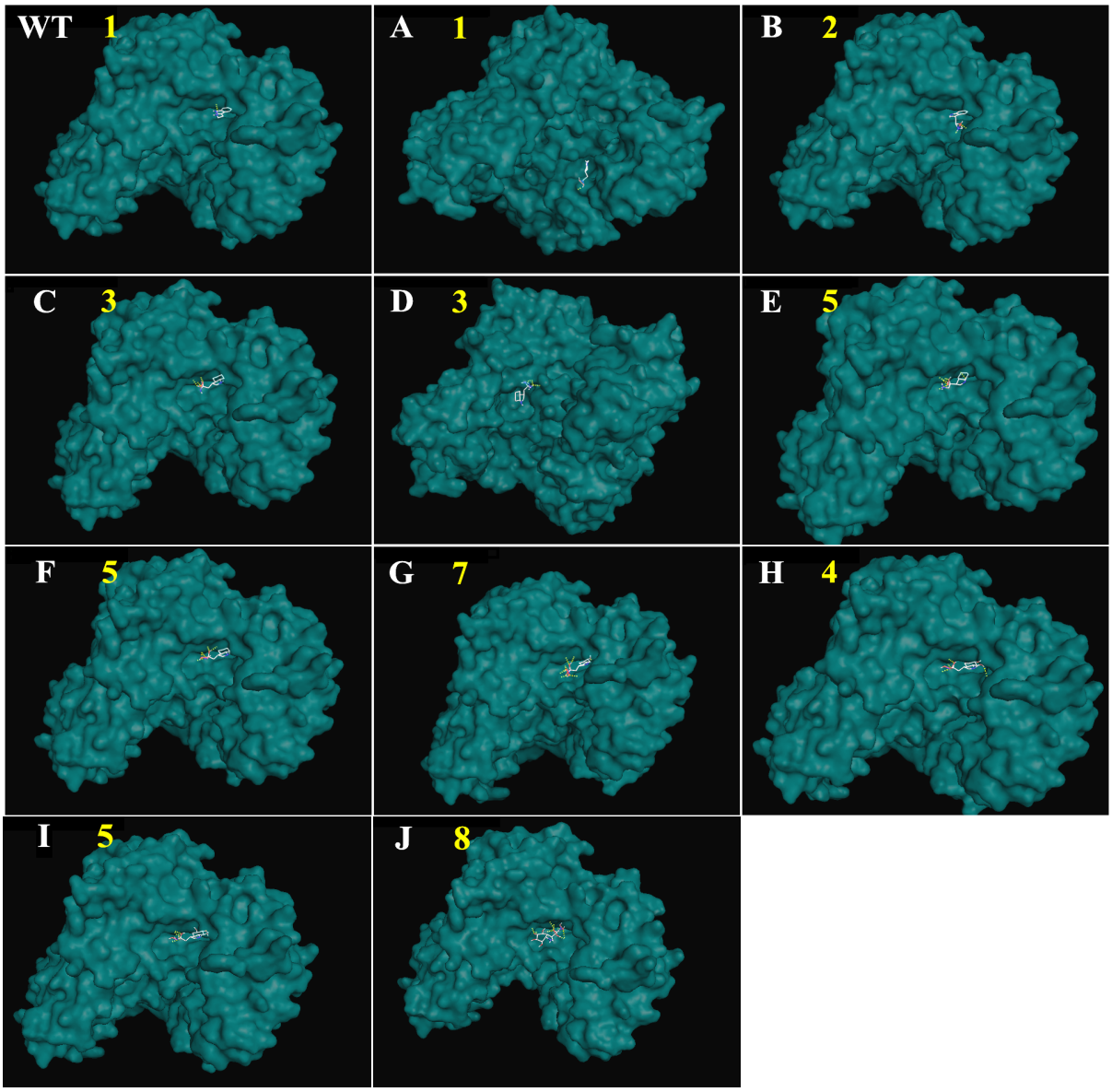

Fig. 17. Molecular models of the strongest binding interactions of the *M. tuberculosis* tryptophan synthase β_2-dimer (green) and **(WT)** wild-type tryptamine, **(A)** 1(2) hydroxyl, **(B)** 2b hydroxyl, **(C)** 2(2)b hydroxyl, **(D)** 2(2)b amine, **(E)** 3(2)b hydroxyl, **(F)** 1(2)+(2)b hydroxyl, **(G)** 1(2)+(2)b+11 hydroxyl, **(H)** 1(2)+(2)b+11+12 hydroxyl, **(I)** 1(2)+(2)b+10+11+12 hydroxyl and **(J)** 1(2)+(2)b+9+10+11+12 hydroxyl. Yellow numbers represent the number of hydrogen bonds in the binding interaction.

strongest binding interactions with the *M. tuberculosis* β_2-dimer, including the hydrogen bonds formed, is presented in Fig. 17. Again, while there were slight discrepancies in the orientation of binding, all ten of the identified tryptamine analogs bound with the highest affinity to the same region of the *M. tuberculosis* β_2-dimer as wild-type tryptamine. Also,

nearly all of the tryptamine analogs increased the number of hydrogen bonds formed with the *M. tuberculosis* β_2-dimer. Again, no analogs decreased the number of hydrogen bonds formed with the β_2-dimer.

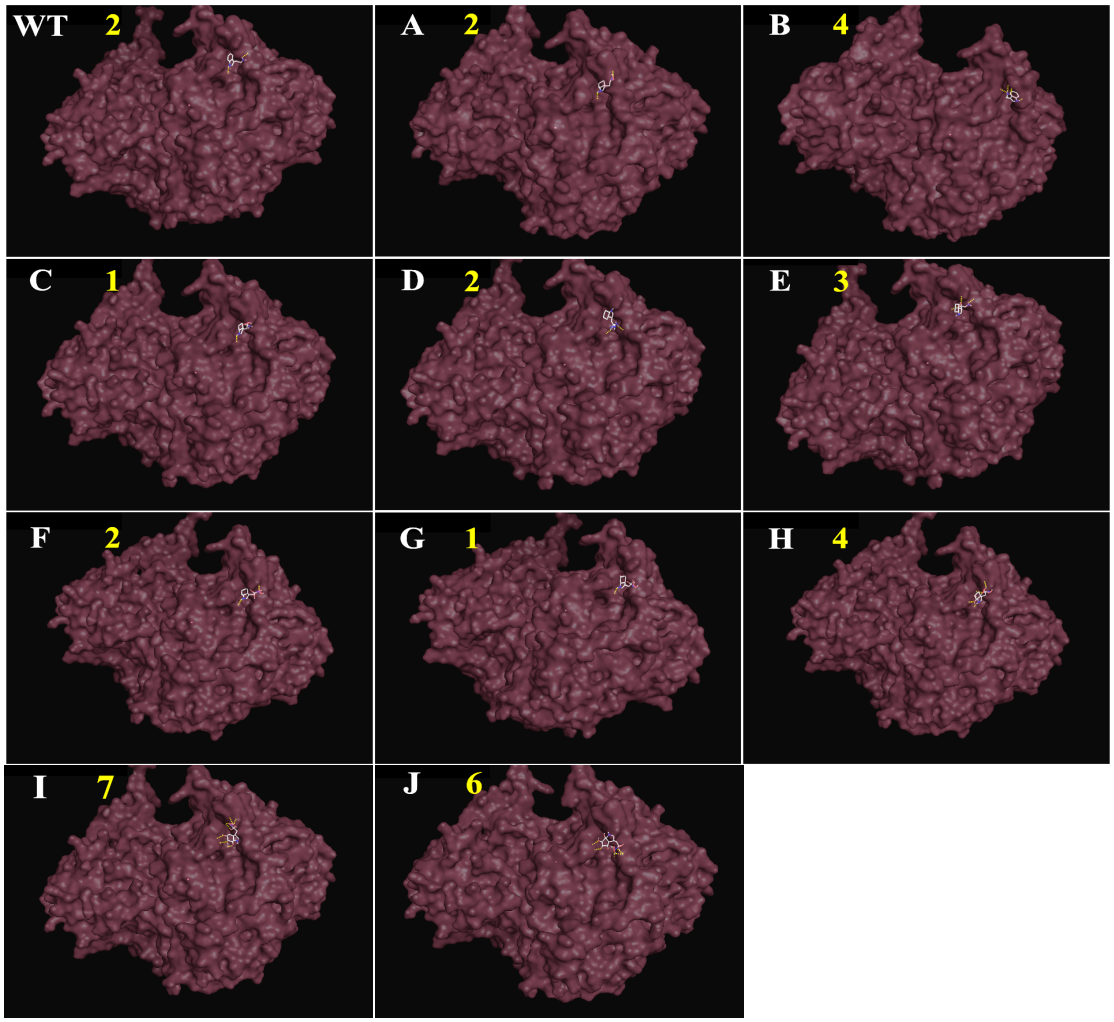

Fig. 18. Molecular models of the strongest binding intereactions of the human MAOB (red) and (**WT)** wild-type tryptamine, **(A)** 1(2) hydroxyl, **(B)** 2b hydroxyl, **(C)** 2(2)b hydroxyl, **(D)** 2(2)b amine, **(E)** 3(2)b hydroxyl, **(F)** 1(2)+(2)b hydroxyl, **(G)** 1(2)+(2)b+11 hydroxyl, **(H)** 1(2)+(2)b+11+12 hydroxyl, **(I)** 1(2)+(2)b+10+11+12 hydroxyl and **(J)** 1(2)+(2)b+9+10+11+12 hydroxyl. Yellow numbers represent the number of hydrogen bonds in the binding interaction.

Finally, we modeled the strongest binding interaction of the ten identified tryptamine analogs to human MAOB. We also vizualized the number of hydrogen bonds that each

tryptamine analog formed with MAOB. The pictures of the tryptamine analogs' strongest binding interactions with the human MAOB, including the hydrogen bonds formed, is presented in Fig. 18. Again, while were slight differences in the orientation of binding, all ten of the identified tryptamine analogs bound with the highest affinity to the same region of human MAOB as wild-type tryptamine. Exactly half of the tryptamine analogs increased the number of hydrogen bonds formed with human MAOB. Two of the analogs decreased the number of hydrogen bonds formed with human MAOB.

Discussion

In silico molecular modeling enabled us to model novel tryptamine analogs and analyze the effects of various functional group additions on the molecules' binding affinities to the following tryptophan synthase subunits: *E. coli* α-subunit, *E. coli* β_2-dimer and the *M. tuberculosis* β_2-dimer. This same molecular modeling process allowed us to determine the effects of these same functional group additions on the molecules' binding affinities to human MAOB. Furthermore, we were able to analyze the location on the enzymes where the ten identified tryptamine analogs bind with the highest affinity. Our research found that additions of certain functional groups to wild-type tryptamine can significantly increase the molecules' tryptophan synthase binding affinities. However, despite the successes in increasing the tryptophan synthase binding affinity in the tryptamine analogs, we could not model any tryptamine-like molecules that both increased tryptophan synthase binding affinity while also decreasing the human MAOB binding affinity. Instead, the ability of the tryptamine analogs to bind to tryptophan synthase was correlated with their ability to bind to human MAOB. This may suggest that tryptophan synthase and human MAOB have similar tryptamine binding sites.

From the molecular modeling of binding regions, we found that the ten tryptamine analogs bind with the highest affinity to the same region as wild-type tryptamine in the *E. coli* tryptophan synthase β_2-dimer, the *M. tuberculosis* β_2-dimer and human MAOB. However, to the *E. coli* tryptophan synthase α-subunit the ten tryptamine analogs bind with the highest affinity to a different region than wild-type tryptamine and there is variance in α-subunit binding regions across the tryptamine analogs. Compared to wild-type tryptamie, the majority of the tryptamine analogs display an increase in the number of hydrogen bonds they

form when bound to the three tryptophan synthase subunits, while only a small number decrease the number of hydrogen bonds in the binding interactions. In the *E. coli* α-subunit one analog increases the number of hydrogen bonds 3.5x compared to wild-type tryptamine, in the *E. coli* β_2-dimer the highest hydrogen bond increase is 4x, and in the *M. tuberculosis* β_2-dimer the greatest hydrogen bond increase is 8x. Similar to the binding affinity assays, half of the tryptamine analogs also display an increase in the number of hydrogen bonds they form with human MAOB when compared to wild-type tryptamine. The other half of the analogs either exhibited the same or a decreased number of hydrogen bonds to human MAOB.

Regardless of the increase in human MAOB binding affinity and an increase in hydrogen bonding to MAOB in half of the analogs, the identified tryptamine analogs show promise as tryptophan synthase inhibitors and subsequently, as novel broad-spectrum antibiotics. Earlier studies have shown that tryptamine and tryptamine-like analogs are potentially successful antibiotics through their inhibition of bacterial tryptophan synthase (Freundlich and Lichestein, 1961; Jianxiong et al., 1997; Okudoh and Wallish, 2012; Faulkner et al., 2016). Our research supports and augments this earlier research by showing that additions of certain functional groups can significantly increase the tryptophan synthase binding affinity, and therefore the molecules' potential as tryptophan synthase inhibitors. Specifically, our data identifies ten potential tryptamine analogs that provide a significant increase from wild-type tryptamine in the bacterial tryptophan synthase binding affinity.

Multiple studies have noted that tryptamine has a high turnover rate in mammalian brains because of MAO-catalyzed oxidative deamination (Yu, 1985; Sullivan et al., 1986). Therefore, the correlated increase in human MAOB binding affinity alongside the increases

in tryptophan synthase binding affinity may affect the potential effectiveness of tryptamine analogs as clinical antibiotic agents. However, further research is required before the effects of MAO on a tryptamine analog antibiotics can be determined. Although the strong MAOB binding affinity of the tryptamine analogs may harm their antibiotic effects, it may introduce another potential pharmaceutical capability. Research has found that certain tryptamine analogs are successful MAO inhibitors *in vivo* (Balsa et al., 1990; Avila et al., 1993). Therefore, given their significant increase in human MAOB binding affinity, the tryptamine analogs identified in this paper display potential as MAO inhibitors. MAO inhibitors are important drugs, utilized in the treatment of Parkinson's disease and other neurological disorders. Moving forward, future research should be dedicated to determining the effectiveness of the tryptamine analogs as MAO inhibitors *in vivo*.

While the tryptamine analogs' potential use as MAO inhibitors is both interesting and intriguing, the primary findings of our paper consists of the identification of ten tryptamine analogs with significant prospects as tryptophan synthase inhibitors and therefore as broad-spectrum antibiotic agents. When compared to wild-type tryptamine, the ten tryptamine analogs exhibited an approximately 18 to 39 times increase in the combined relative binding affinity across the three tryptophan synthase subunits tested in this study. Furthermore, although there was a correlation between increases in both the tryptophan synthase and MAOB binding affinities, this was not a 1:1 relationship. Instead, our identified tryptamine analogs displayed a much greater increase in tryptophan synthase subunit binding affinity than in MAOB binding affinity. The analogs' increases in hydrogen bond formation to the tryptophan synthase subunits when compared to wild-type tryptamine also supports the quantitative increases in binding affinity because increasing hydrogen bonding interactions

should increase the binding affinity of the protein-ligand complex. This data indicates significant potential for *in vivo* tryptophan synthase inhibition. Significantly increased binding affinities to the tryptophan synthase subunits may correlate to increased inhibition of the tryptophan synthase enzyme and subsequently increased antibiotic capabilities.

Additionally, the similarities in binding sites to the *E. coli* and *M. tuberculosis* β_2-dimers between wild-type tryptamine and the tryptamine analogs provide further evidence towards the analogs' potential as tryptophan synthase inhibitors and antibiotic agents. The similarities in binding region suggest that the tryptamine analogs will have a similar inhibitory effect on tryptophan synthase as wild-type tryptamine. However, the analogs did bind to different regions on the *E. coli* α-subunit than wild-type tryptamine. While these differences in binding sites may affect the functionality of the tryptamine analogs, this cannot be guaranteed until the analogs are synthesized and tested further.

Following this research, we filed a provisional patent on the novel structures of the ten identified tryptamine analogs. If these tryptamine analogs are successful as tryptophan synthase inhibitors, as the *in silico* data suggests, they may succeed as antibiotic agents. Consequently, these novel molecules may provide new antibiotic drugs to combat the growing global health crisis of antibiotic resistance and help fill the discovery void of the past three decades. Furthermore, given past research that identified tryptophan synthase inhibitors as potential antibiotic adjuvants, these tryptamine molecules may also facilitate the success of other clinical antibiotics (Hirakawa et al., 2004). Therefore, we propose that the ten tryptamine analogs presented in Fig. 14 are deserving of synthesis and further *in vitro* and *in vivo* research into their antibiotic properties.

Works Cited

Avila, M., Balsa, M.D., Fernandez-Alvarez, E., Tipton, K.F., and Unzeta, M. (1993). The effect of side chain substitution at positions 2 and 3 of the heterocyclic ring of N-acetylenic analogues of tryptamine as monoamine oxidase inhibitors. Biochemical Pharmacology 45, 2231–2237.

Balsa, D., Fernandez-Alvarez, E., Tipton, K.F., and Unzeta, M. (1990). Inhibition of MAO by substituted tryptamine analogues. In Amine Oxidases and Their Impact on Neurobiology, (Springer, Vienna), pp. 103–105.

Barends, T.R.M., Dunn, M.F., and Schlichting, I. (2008). Tryptophan synthase, an allosteric molecular factory. Curr Opin Chem Biol 12, 593–600.

Bartlett, J.G., Gilbert, D.N., and Spellberg, B. (2013). Seven Ways to Preserve the Miracle of Antibiotics. Clin Infect Dis 56, 1445–1450.

Bax, R., and Griffin, D. (2012). Introduction to Antibiotic Resistance. 1–12.

Binda, C., Newton-Vinson, P., Hubálek, F., Edmondson, D.E., and Mattevi, A. (2002). Structure of human monoamine oxidase B, a drug target for the treatment of neurological disorders. Nat Struct Mol Biol 9, 22–26.

Boucher, H.W., Talbot, G.H., Bradley, J.S., Edwards, J.E., Gilbert, D., Rice, L.B., Scheld, M., Spellberg, B., and Bartlett, J. (2009). Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America. Clin Infect Dis 48, 1–12.

Brown, P.D. (2010). Multiple Drug Resistance. In Multiple Drug Resistance, (Nova Science Publishers Inc.),.

CDC (2013). Antibiotic Resistance Threats in the United States, 2013 (US Department of Health and Human Services).

Chaudhary, K., and Roos, D.S. (2005). Protozoan genomics for drug discovery. Nat Biotech 23, 1089–1091.

Chemical Sciences Roundtable. (2014). Technological Challenges in Antibiotic Discovery and Development: : A Workshop Summary. The National Academies Press.

Dallakyan, S., and Olson, A.J. (2015). Small-molecule library screening by docking with PyRx. Methods Mol. Biol. 1263, 243–250.

Daniels, R. (2012). Antibiotic resistance: a crisis in the making. Emergency Nurse 20, 11–11.

Davies, J. (2006). Where have All the Antibiotics Gone? Can J Infect Dis Med Microbiol 17, 287–290.

Davies, J., and Davies, D. (2010). Origins and Evolution of Antibiotic Resistance. Microbiol Mol Biol Rev 74, 417–433.

Dias, M.V.B., Canduri, F., Silveira, N.J.F. da, Czekster, C.M., Basso, L.A., Palma, M.S., Santos, D.S., and Azevedo, W.F. de (2006). Molecular models of tryptophan synthase

from Mycobacterium tuberculosis complexed with inhibitors. Cell Biochem Biophys 44, 375–384.

Fatmi, M.Q., Ai, R., and Chang, C.A. (2009). Synergistic Regulation and Ligand-Induced Conformational Changes of Tryptophan Synthase. Biochemistry 48, 9921–9931.

Faulkner, K.C., Hurley, K.A., and Weibel, D.B. (2016). 5-Alkyloxytryptamines are membrane-targeting, broad-spectrum antibiotics. Bioorganic & Medicinal Chemistry Letters 26, 5539–5544.

Finn, J., Langevine, C., Birk, I., Birk, J., Nickerson, K., and Rodaway, S. (1999). Rational herbicide design by inhibition of tryptophan biosynthesis. Bioorganic & Medicinal Chemistry Letters 9, 2297–2302.

Fleming, A. (1945). Penicillin.

Fleming, A. (1946). Penicillin's finder assays its future. New York Times.

Freundlich, M., and Lichstein, H.C. (1962). TRYPTOPHANASE-TRYPTOPHAN SYNTHETASE SYSTEMS IN ESCHERICHIA COLI I. J Bacteriol 84, 979–987.

Gottfried, J. (2005). History Repeating? Avoiding a Return to the Pre-Antibiotic Age. Third Year Paper. Harvard Law School.

Gross, M. (2013). Antibiotics in crisis. Current Biology 23, R1063–R1065.

Hersh, A.L., Newland, J.G., Beekmann, S.E., Polgreen, P.M., and Gilbert, D.N. (2012). Unmet Medical Need in Infectious Diseases. Clin Infect Dis 54, 1677–1678.

Hirakawa, H., Inazumi, Y., Masaki, T., Hirata, T., and Yamaguchi, A. (2005). Indole induces the expression of multidrug exporter genes in Escherichia coli. Molecular Microbiology 55, 1113–1126.

Humphrey, W., Dalke, A., and Schulten, K. (1996). VMD - Visual Molecular Dynamics. H Molec. Graphics 14, 33–38.

Jianxiong, L., Genhui, C., and Webster, J.M. (1997). SYNTHESIS AND ANTISTAPHYLOCOCCAL ACTIVITY OF NEMATOPHIN AND ITS ANALOGS. Bioorganic & Medicinal Chemistry Letters 7, 1349–1352.

Kachalova, G.S., Burenkov, G.P., Strizhov, I.N., Svergun, I.D., and Bartunik, H.D. (To Be Published). Structure Tryptophan Synthase beta subunit dimer from Mycobacterium Tuberculosis.

Katz, M.L., Mueller, L.V., Polyakov, M., and Weinstock, S.F. (2006). Where have all the antibiotic patents gone? Nat Biotech 24, 1529–1531.

Lushniak, B.D. (2014). Antibiotic Resistance: A Public Health Crisis. Public Health Reports 129, 314–316.

Luyt, C.-E., Bréchot, N., Trouillet, J.-L., and Chastre, J. (2014). Antibiotic stewardship in the intensive care unit. Critical Care 18, 480.

Manfredi, R. (2009). The Phenomenon of Antibiotic Resistance in an Evolutionary Perspective. In Antibiotic Resistance : Causes and Risk Factors, Mechanisms and Alternatives,.

Medina, E., and Pieper, D.H. (2016). Tackling Threats and Future Problems of Multidrug-Resistant Bacteria. 3–33.

Miles, E.W. (2009). Tryptophan Synthase: Structure, Function and Subunit Interaction. In Advances in Enzymology and Related Areas of Molecular Biology, (John Wiley & Sons),.

Morrill, H.J., and LaPlante, K.L. (2015). Overconsumption of antibiotics. The Lancet Infectious Diseases 15, 377–378.

Neu, H.C. (1992). The Crisis in Antibiotic Resistance. Science 257, 1064–1073.

Nishio, K., Morimoto, Y., Ishizuka, M., Ogasahara, K., Tsukihara, T., and Yutani, K. (2005). Conformational Changes in the α-Subunit Coupled to Binding of the β2-Subunit of Tryptophan Synthase from Escherichia coli: Crystal Structure of the Tryptophan Synthase α-Subunit Alone,. Biochemistry 44, 1184–1192.

Nishio, K., Ogasahara, K., Morimoto, Y., Tsukihara, T., Lee, S.J., and Yutani, K. (2010). Large conformational changes in the Escherichia coli tryptophan synthase β2 subunit upon pyridoxal 5′-phosphate binding. FEBS Journal 277, 2157–2170.

Nowell, H., Attfield, J.P., and Cole, J.C. (2002). 195802: Experimental Crystal Structure Determination. The Cambridge Crystallographic Data Centre.

Okudoh, V.I., and Wallis, F.M. (2007). Antimicrobial activity of rare actinomycetes isolated from natural habitats in KwaZulu-Natal, South Africa. South African Journal of Science 103, 216–222.

Okudoh, V.I., and Wallis, F.M. (2012). Enhanced Recovery and Identification of a Tryptamine-Related Antibiotic Produced by Intrasporangium N8 from KwaZulu-Natal, South Africa. Tropical Journal of Pharmaceutical Research 11, 729–737.

O'Neill, J. (2016). Tackling Drug-Resistant Infections Globally: Final Reports and Recommendations (Review on Antimicrobial Resistance).

R Core Team (2016). R: A Language and Environment for Statistical Computing (Vienna, Austria: R Foundation for Statistical Computing).

Ren, D., Bedzyk, L.A., Ye, R.W., Thomas, S.M., and Wood, T.K. (2004). Stationary-phase quorum-sensing signals affect autoinducer-2 and gene expression in Escherichia coli. Appl. Environ. Microbiol. 70, 2038–2043.

Sarker, K.D., and Hardman, J.K. (1995). Affinities of phosphorylated substrates for the E. coli tryptophan synthase alpha-subunit: roles of Ser-235 and helix-8' dipole. Proteins 21, 130–139.

Schneider, T.R., Gerhardt, E., Lee, M., Liang, P.-H., Anderson, K.S., and Schlichting, I. (1998). Loop Closure and Intersubunit Communication in Tryptophan Synthase,. Biochemistry 37, 5394–5406.

Silver, L.L. (2007). Multi-targeting by monotherapeutic antibacterials. Nat Rev Drug Discov 6, 41–55.

Spellberg, B., and Gilbert, D.N. (2014). The Future of Antibiotics and Resistance: A Tribute to a Career of Leadership by John Bartlett. Clin Infect Dis 59, S71–S75.

Sullivan, J.P., McDonnell, L., Hardiman, O.M., Farrell, M.A., Phillips, J.P., and Tipton, K.F. (1986). The oxidation of tryptamine by the two forms of monoamine oxidase in human tissues. Biochemical Pharmacology 35, 3255–3260.

Van Boeckel, T.P., Gandra, S., Ashok, A., Caudron, Q., Grenfell, B.T., Levin, S.A., and Laxminarayan, R. (2014). Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. The Lancet Infectious Diseases 14, 742–750.

Ventola, C.L. (2015). The Antibiotic Resistance Crisis. P T 40, 277–283.

Viswanathan, V.K. (2014). Off-label abuse of antibiotics by bacteria. Gut Microbes 5, 3–4.

Wang, D., Ding, X., and Rather, P.N. (2001). Indole can act as an extracellular signal in Escherichia coli. J. Bacteriol. 183, 4210–4216.

White, R.J. (2012). The Early History of Antibiotic Discovery: Empiricism Ruled. 3–31.

Wickham, H. (2009). ggplot2: Elegant Graphics for Data Analysis (Springer-Verlag New York).

Winzer, K., Hardie, K.R., and Williams, P. (2002). Bacterial cell-to-cell communication: sorry, can't talk now - gone to lunch! Curr. Opin. Microbiol. 5, 216–222.

Wright, G.D. (2012). The Origins of Antibiotic Resistance. 13–30.

Yu, P.H. (1985). Some Aspects of the Oxidative Deamination of Tryptamine by MAO-A and MAO-B in Different Tissues. In Neuropsychopharmacology of the Trace Amines, A.A. Boulton, L. Maitre, P.R. Bieck, and P. Riederer, eds. (Humana Press), pp. 301–308.

(2017). ChemDraw Professional (PerkinElmer Informatics).

The PyMOL Molecular Graphics System (Schrödinger, LLC).

Appendices

Provisional Patent Application Receipt

Electronic Acknowledgement Receipt	
EFS ID:	28974537
Application Number:	62487461
International Application Number:	
Confirmation Number:	9014
Title of Invention:	Structurally Novel Tryptamine Analogs and Methods of Treatment of Bacterial Infection through Inhibition of Tryptophan Synthase
First Named Inventor/Applicant Name:	Jared Schattenkerk
Correspondence Address:	OC Neuroscience - 4000 Barranca Parkway, Suite 250 - Irvine CA 92604 US 805-585-9752 ocneuroscience@outlook.com
Filer:	Aaron McMurtrey
Filer Authorized By:	
Attorney Docket Number:	
Receipt Date:	19-APR-2017
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Time Stamp:	22:19:29
Application Type:	Provisional

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$130

RAM confirmation Number	042017INTEFSW22210600
Deposit Account	
Authorized User	

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Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Specification	Specification_File_Schattenkerk.pdf	272927	no	43
Warnings:					
Information:					
2	Drawings-other than black and white line drawings	Figure_1.pdf	201437	no	1
Warnings:					
Information:					
3	Drawings-only black and white line drawings	Figure_2.pdf	155565	no	1
Warnings:					
Information:					
4	Drawings-only black and white line drawings	Figure_3.pdf	179702	no	1
Warnings:					
Information:					
5	Drawings-only black and white line drawings	Figure_4.pdf	178658	no	1
Warnings:					
Information:					

6	Drawings-only black and white line drawings	Figure_5.pdf	188822	no	1
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7	Drawings-other than black and white line drawings	Figure_6.pdf	186189	no	1
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8	Drawings-other than black and white line drawings	Figure_7.pdf	199870	no	1
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9	Drawings-other than black and white line drawings	Figure_8.pdf	243651	no	1
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10	Drawings-other than black and white line drawings	Figure_9.pdf	274510	no	1
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11	Drawings-other than black and white line drawings	Figure_10.pdf	245788	no	1
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12	Drawings-other than black and white line drawings	Figure_11.pdf	256338	no	1
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13	Drawings-other than black and white line drawings	Figure_12.pdf	252963	no	1
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14	Drawings-other than black and white line drawings	Figure_13.pdf	260020	no	1
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15	Drawings-other than black and white line drawings	Figure_14.pdf	267095	no	1
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16	Drawings-only black and white line drawings	Figure_15.pdf	244874	no	1
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17	Drawings-other than black and white line drawings	Figure_16.pdf	241389	no	1
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19	Drawings-other than black and white line drawings	Figure_18.pdf	243230	no	1
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20	Drawings-other than black and white line drawings	Figure_19.pdf	248704	no	1
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21	Provisional Cover Sheet (SB16)	Provisional_Patent_Application_Cover_Sheet_Schattenkerk.pdf	306148	no	3
Warnings:					
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22	Application Data Sheet	Application_data_sheet_Schattenkerk.pdf	1822933	no	8
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